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HISTOLOGY OF THE CARYOPSIS OF YELLOW DENT CORN, WITH REFERENCE TO RESISTANCE AND SUSCEPTIBILITY TO KERNEL ROTS [1]

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INTRODUCTION

Inasmuch as the kernel of corn (*Zea mays* L.) is susceptible to a number of ear rot organisms, it has seemed advisable to obtain a picture of the histology of the normal spikelet and kernel as a basis for the study of various phases of the disease problem. This paper, therefore, outlines briefly the development and the microscopic anatomy of the caryopsis of dent corn and is concerned primarily with those structures that may have a bearing on the problem of host resistance or susceptibility to fungus diseases in general and to invasion by *Diplodia zeae* (Schw.) Lév. in particular.

MATERIAL AND METHODS

The hand-pollinated yellow dent corn used in this study was obtained from the plots of the departments of plant pathology and genetics at the University of Wisconsin and from J. R. Holbert, of Bloomington, Ill. Kernels both from strains that had shown themselves over a period of years to be resistant to ear rots in the field and from those that under comparable conditions had proved susceptible were collected during the summers of 1930 to 1933, inclusive. The first collections were made 2 days after pollination. Specimens were taken at intervals of from 2 to 7 days during the earlier periods of development of the kernel, killed in medium chromo-acetic solution, formol acetic alcohol, or Gilson's or Juel's zinc chloride fixative, and embedded in paraffin by the alcohol-chloroform method.[3] Before fixation, the healthy mature kernels were soaked in water overnight, and when softened the sides or distal ends of the kernels were trimmed so as to expose portions of the starchy endosperm to facilitate the penetration of the various fluids.

A simple method of overcoming the difficulty usually experienced in sectioning kernels approaching maturity is to soak the embedded material in water for from a few hours to overnight, after having trimmed the paraffin block in such a manner as to expose the tissue

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[3] The material embedded during the four seasons included specimens from inbred strains A, A48, A9, A956, Br10, R4, R313, L, and Lan; single crosses nos. 365, 48, and 58; and top crosses Krug × A48 and Krug × Lan.

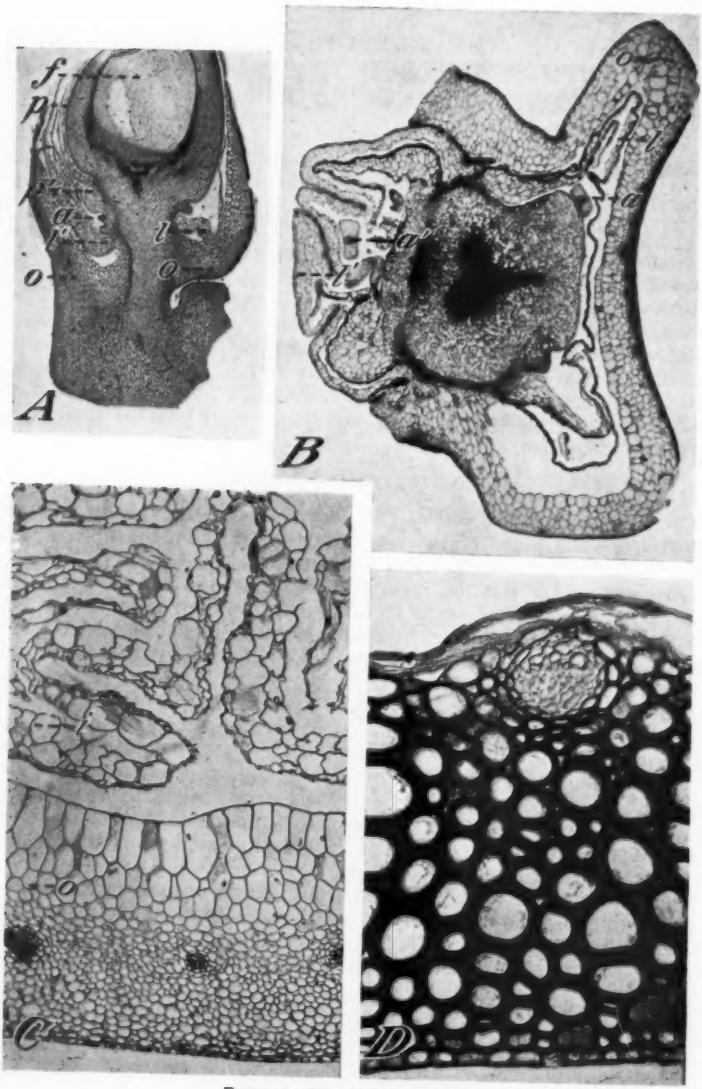


FIGURE 1.—The pistillate spikelet.

FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE

on the side to be cut. Seldom, however, can an entire kernel be successfully sectioned at one time, hence intermittent soaking and cutting are usually necessary. The sections were cut to a thickness of 8μ to 10μ. This method provided better sections than were obtained by the use of a hydrofluoric acid treatment as recommended for wheat kernels in an earlier paper (*7*).[4]

The principal stains used were Flemming's triple combination or safranine and light green following either chromo-acetic or formol acetic alcohol fixative in the case of healthy kernels, and Delafield's haematoxylin, safranine, and orange G after Gilson's or Juel's fixative when dealing with infected kernels. Sudan III served to indicate the presence of suberized membranes. In case of a positive reaction to this stain, insolubility of the parts in question in 72-percent H_2SO_4 was considered additional evidence of the presence of suberinlike substances.

The illustrations presented are unretouched photographs and photomicrographs.

HISTOLOGY OF CORN KERNEL

PISTILLATE SPIKELET

It is well known that the pistillate spikelet of dent corn consists of two flowers, the basal of which is usually rudimentary and nonfunctional. The aborted flower contains a rudimentary pistil and three rudimentary stamens enclosed by two thin folded glumes, the palet and lemma. In the fertile flower there are rudimentary stamens and a functional ovary, also encircled by palet and lemma, the whole being surrounded by the heavier outer glumes (fig. 1, *A*, *B*, *C*, *D*). These glumes constitute the chaff of the cob and mature grain.

At flowering time, the ovary wall is composed of a number of rows of thin-walled cells containing considerable starch, an outer epidermal layer, and a somewhat thinner-walled inner epidermis. Two strands from the vascular elements of the pedicel traverse the wall on the germinal side of the ovary and pass into the two lobes of the style. In the style there is also a group of nonvascular cells that parallels each vascular bundle on its inner side. At the top of the ovary these groups of cells leave their positions along the vascular bundles, cross the ovary wall, and terminate on its inner surface near the top of the ovular cavity (fig. 2, *A*, *B*), providing a pathway along which the pollen tube travels to the cavity of the ovary. Miller (*6, p. 258*) speaks of them as "sheathlike" cells and Randolph (*8*) finds an explanation for their presence in the manner in which the three carpels have become fused to form the compound pistil. She considers the strands to be composed largely of epidermal cells. The

[4] Reference is made by number (italic) to Literature Cited, p. 882.

EXPLANATORY LEGEND FOR FIGURE 1

A, Longitudinal section of spikelet, fixed 2 days after pollination: *f*, Ovary of fertile flower encircled by palet (*p*) and lemma (*l*); *a*, aborted flower enclosed by palet (*p'*) and lemma (*l'*); *o*, outer glumes. Stained with triple stain. × 8. *B*, Cross section through a spikelet at the level of the aborted flower shown in *A*, *a*. The outer glume subtending the aborted flower has been removed. Spikelet fixed 7 days after pollination: *o*, Outer glume; *l*, lemma of fertile flower; *l'*, lemma of aborted flower; *a* and *a'*, rudimentary anthers of fertile and aborted flowers. Stained with triple stain. × 10. *C*, Portion of a cross section of a spikelet, fixed 2 days after pollination, showing the structure of the outer glume (*o*) and the structure and folding of the inner glumes (*i*). Stained with triple stain. × 54. *D*, Portion of a cross section of an outer glume, fixed 27 days after pollination. The walls of the cells beyond the vascular elements have become much thickened; those within the line of bundles have collapsed, leaving the vascular elements in a position near the inner margin of the glume. Stained with triple stain. × 136.

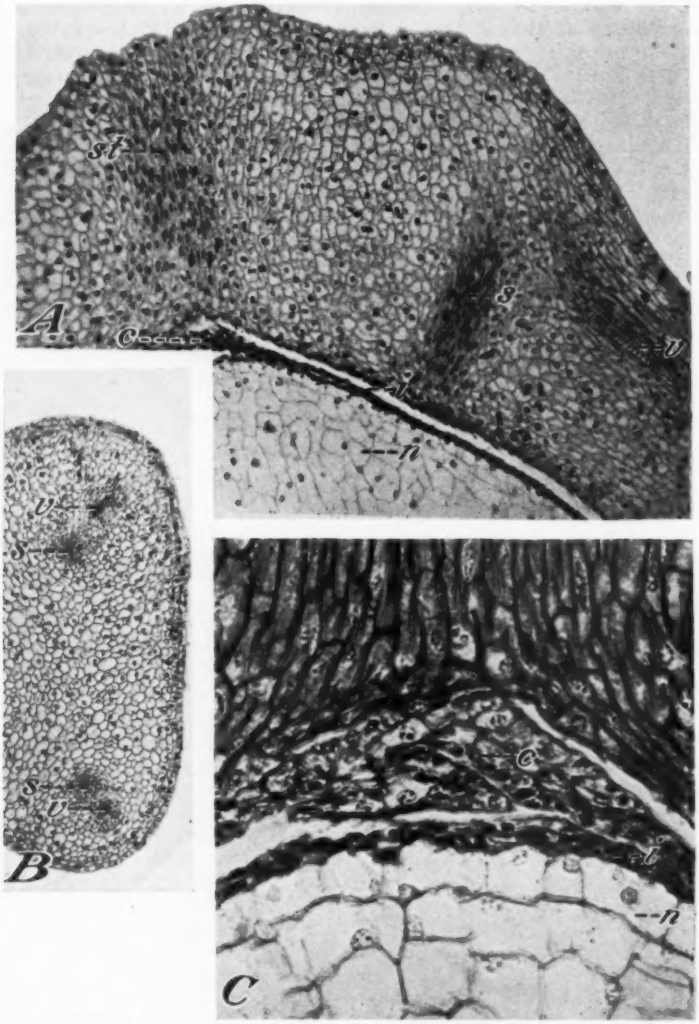


FIGURE 2.—Sections from the top of the ovary. *A*, A portion of a longitudinal section of an ovary, fixed 2 days after pollination. The ovary wall is cut in a plane that includes parts of the stylar canal and of the group of "sheathlike" cells, along which the pollen tube travels to the cavity of the ovary, and one of the two vascular strands that extend into the silk. *st*, Stylar canal; *s*, sheathlike cells; *v*, vascular elements; *c*, cone-shaped thickening of the outer integument at the base of the stylar canal; *i*, inner integument; *n*, nucellus. Stained with safranine and light green. × 120. *B*, Portion of a cross section of the ovary, showing the relative positions of the vascular elements and the sheathlike cells in the ovary wall near the base of the style: *v*, Vascular elements; *s*, sheathlike cells. Stained with triple stain. × 70. *C*, Detail of the cone-shaped folding of the outer integument at the base of the stylar canal. This section is from the ovary shown in *A*; *c*, *i*, and *n* as in *A*. Stained with safranine and light green. × 450.

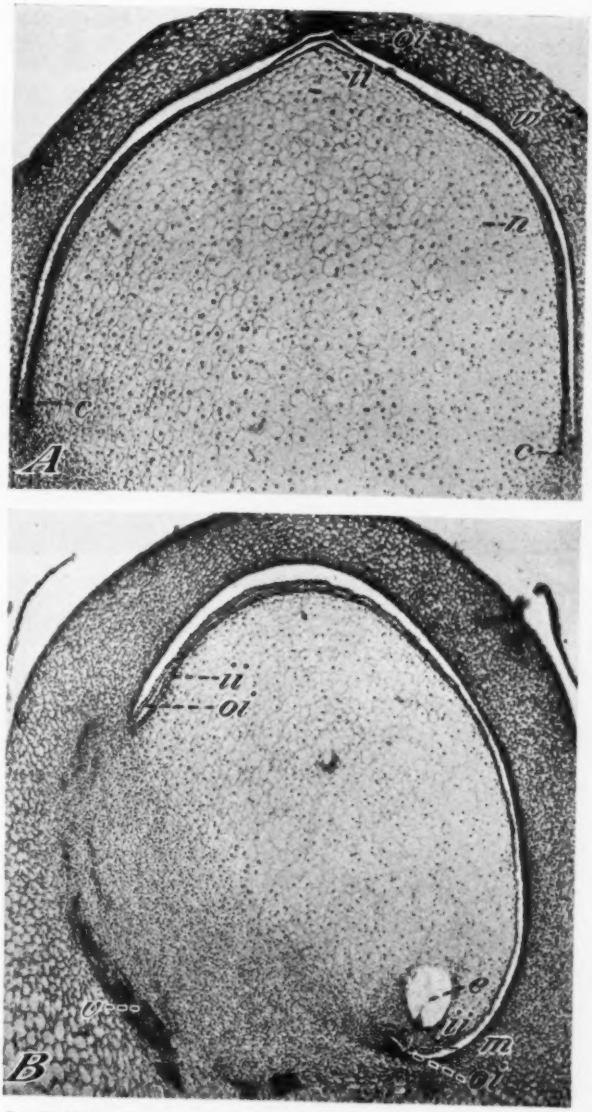


FIGURE 3.—Longitudinal sections of ovaries, fixed 2 days after pollination. *A*, Section cut parallel to the germinal side of the ovary; *oi*, outer integument, showing the cone-shaped protuberance that projects into the depression of the stylar canal; *ii*, inner integument; *w*, wall of ovary; *n*, nucellus; *c*, chalaza. Stained with triple stain. × 50. *B*, Near-longitudinal section cut approximately at right angles to *A*; *ii*, inner integument; *oi*, outer integument; *e*, embryo sac; *m*, micropyle; *v*, vascular elements. Stained with safranine and light green. × 42.

modified campylotropous ovule is attached for approximately one-third of its surface to the bottom of the cavity of the ovary (fig. 3).

The integuments of the ovule are layers of thin-walled tissue for the most part two cells thick, except at the micropyle, where the

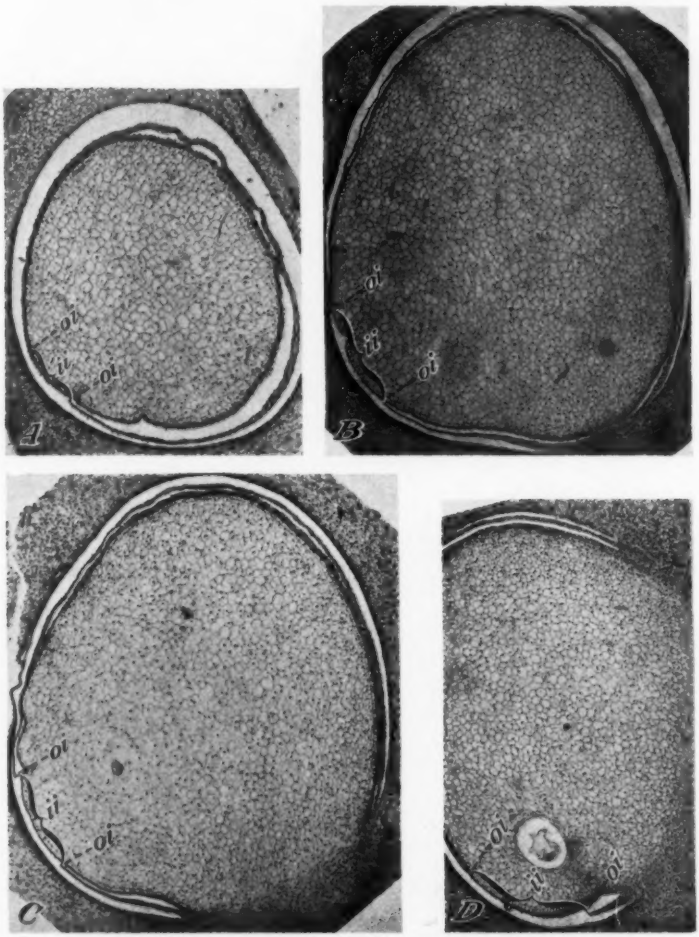


FIGURE 4.—Cross sections from a single ovary, showing the extent of the outer integument and the exposed surface of the inner integument at four different levels. *A*, Near the top of the ovary; *B*, at the equator; *C*, through the antipodal cells of the embryo sac; *D*, in the region of the fertilized egg cell. *oi*, Ends of outer integument; *ii*, surface of inner integument not covered by outer integument. Stained with triple stain. × 35.

inner integument especially becomes somewhat club-shaped in section. Neither the integuments nor the nucellus contain any starch, though at the stage of development shown in figures 3 and 4 the cells of the ovary wall and the pedicel of the floret possess an abundance of that

food material (figs. 6, *B*, and 15, *A*). The inner integument covers the free surface of the ovule completely with the exception of the micropylar orifice and usually is in contact with the epidermis of the

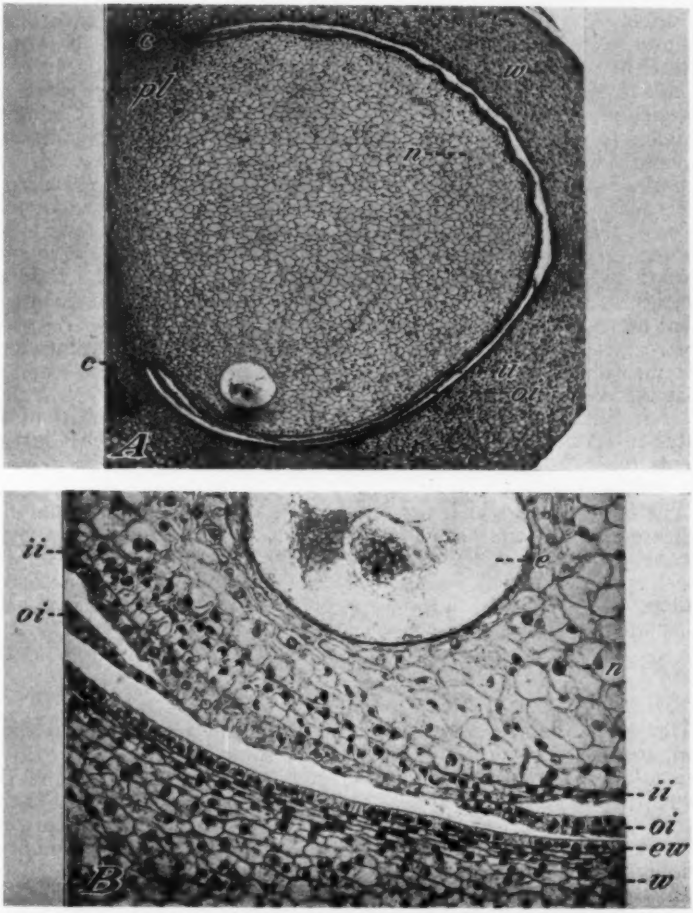


FIGURE 5.—Cross section of an ovary, showing the tissues in the region of the embryo sac. *A*, Ovary fixed 2 days after pollination, showing, in the absence of a funiculus, the similarity at that stage of development between the nucellar cells and the placental tissue of the ovary wall; *c*, chalaza; *pl*, placental tissue; *w*, wall of ovary; *n*, nucellus; *ii*, inner integument; *oi*, outer integument. Stained with triple stain. × 42. *B*, Detail of *A* in the region of the embryo sac, showing the extent of the outer integument in this ovary; *ii*, inner integument; *oi*, outer integument; *e*, embryo sac; *n*, nucellus; *ew*, inner epidermis of the ovary wall (*w*). × 205.

nucellus. The outer integument, which originates alongside of the inner integument, occupies a parallel position throughout its extent except for a cone-shaped folding which projects into the depression of the so-called "stylar canal" at the distal end of the ovary (figs. 2, *C*; 3, *A*, *B*).

Concerning the extent of the outer integument, several writers have shown considerable unanimity of opinion. True (*10, p. 215*) states that "The outer integument is incomplete, failing to cover an area extending from the micropyle to the base of the style, in length, and in width equal to about one-half the diameter of the ovule." Weatherwax (*11, p. 491*) says: "The one part of the outer integument seldom grows further than the top of the ovary, where it forms a folded or wedge-shaped body closing the stylar canal." Miller (*6, p. 258*) states that "the outer coat of the ovule is incomplete and extends about half way around it", and Haddad (*3, p. 7*) is of the opinion that "the outer integument extends only a short distance beyond the stylar canal where it terminates."

Serial longitudinal and cross sections of entire ovaries, prepared in the course of this study, indicate that the outer integument covers more of the surface of the inner integument than the statements just quoted might suggest (figs. 4, 5, 6, *B*). Although there were some variations in the extent of the outer integument in the different ovaries, in all cases examined the exposed surface of the inner integument appeared to be a comparatively narrow, more or less triangular area. The apex of the triangle was to be found near the point where the sheathlike cells of the style terminate in the ovary wall and its base below the micropyle. In sections fixed as early as 2 or 3 days after fertilization, it is often difficult to determine the limits of the outer integument because of degenerative changes in both integuments which result in the two structures appearing to be more or less unified.

The nucellus, in which the embryo sac is embedded, consists of a delicate parenchymatous tissue bordered on its free surface by a distinct but thin-walled epidermal layer. Before fertilization the nucellus and what may be called the funicular-hilar-placental region—although there is neither funiculus nor true hilum—merge into each other without any distinctly marked cell differences (fig. 5, *A*).

DEVELOPMENT OF KERNEL

Embryo

Development of the kernel begins almost immediately after fertilization, and growth proceeds rapidly. According to Miller (*6*), fertilization takes place 26 to 28 hours after pollination, and traces of the pollen tube may be visible within the embryo sac for some time thereafter. He notes the rapid growth of the endosperm and states that the cells of the endosperm may fill the embryo sac when the embryo numbers only 14 to 16 cells.

In sections of spikelets fixed by the writer 2 days after pollen had been applied to the silk of the ear, the pollen tube, staining a deep red with safranine, was visible in the micropyle and embryo sac. In most cases fertilization had taken place. Sections of slightly older spikelets indicated that division of the endosperm nucleus had begun almost immediately after fertilization; they also showed numerous free nuclei formed before cell walls appeared and a more rapid division of the endosperm than of the egg nucleus (fig. 6, *A*). When the developing endosperm had almost filled the enlarged nucellar space the embryo still remained comparatively small (fig. 7, *C*), although in a nearly mature caryopsis it occupies approximately one-third to one-half of the surface of a median longitudinal section of a kernel (fig. 8).

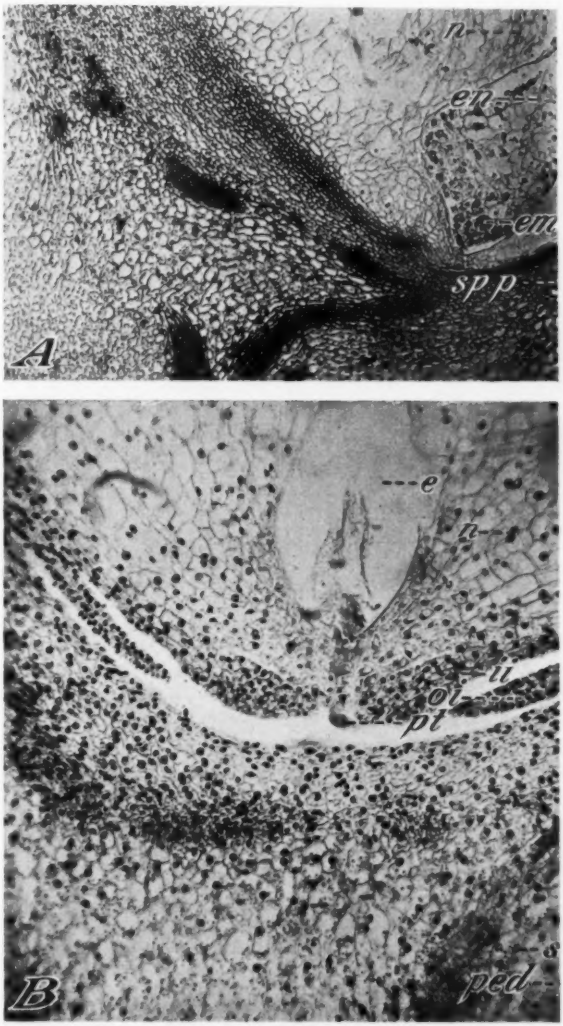


FIGURE 6.—Portions of longitudinal sections of developing kernels, showing tissues in the regions of the embryo. *A*, Portion of a longitudinal section of a developing kernel, showing the more rapid growth of the endosperm than of the embryo. Strain A, grown at Madison, Wis.; open-pollinated; fixed on August 15, at which time the silks were still green: *n*, Nucellus; *en*, endosperm; *em*, embryo; *sp. p*, spongy pericarp. Stained with triple stain. × 48. *B*, Portion of a longitudinal section of an ovary, showing the relative positions of the two integuments at the micropyle. Strain A48, grown at Madison, Wis.; fixed on August 15, 2 days after pollination: *e*, Embryo sac; *n*, nucellus; *ii*, inner integument; *oi*, outer integument; *pt*, pollen tube; *s*, starch granules; *ped*, pedicel. Stained with triple stain. × 125.

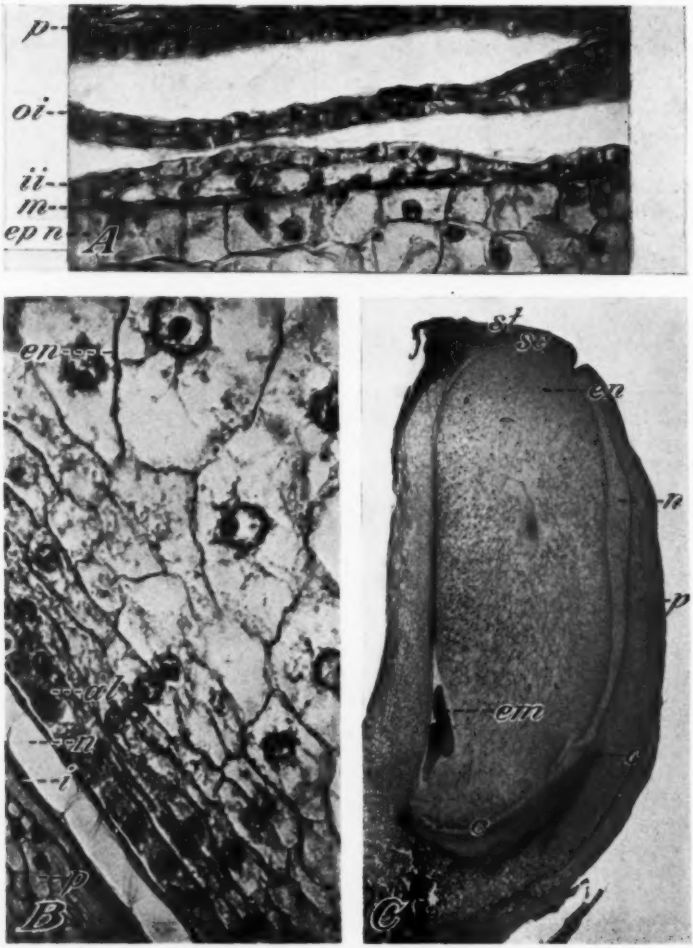


FIGURE 7.—Developmental phases of the corn kernel. *A*, Portion of a cross section of a developing kernel of strain A48, fixed 5 days after pollination, showing degenerative changes in both integuments: *ep. n*, Epidermis of nucellus; *m*, line along which the suberized membranes of the testa will appear; *ii*, inner integument; *oi*, outer integument; *p*, pericarp. Stained with triple stain. × 393. *B*, Detail of *C*. A portion of the endosperm between the embryo and style. At this stage of development, cell division seems to be more active in this region than in other parts of the endosperm; little starch is to be found in the endosperm. *en*, Endosperm; *al*, aleurone layer (immature); *n*, remains of nucellus; *i*, remnant of the integuments; *p*, pericarp. Stained with triple stain. × 203. *C*, Longitudinal section, showing the relative sizes of the embryo and endosperm in a developing kernel of strain A, open-pollinated, fixed on August 23 at Madison, Wis.: *st*, Remnant of the style; *sc*, stylar canal; *en*, endosperm; *n*, remnant of the nucellus; *p*, pericarp; *em*, embryo; *c*, chalaza; *c-c*, hilar opening through the integument. Stained with triple stain. × 13.

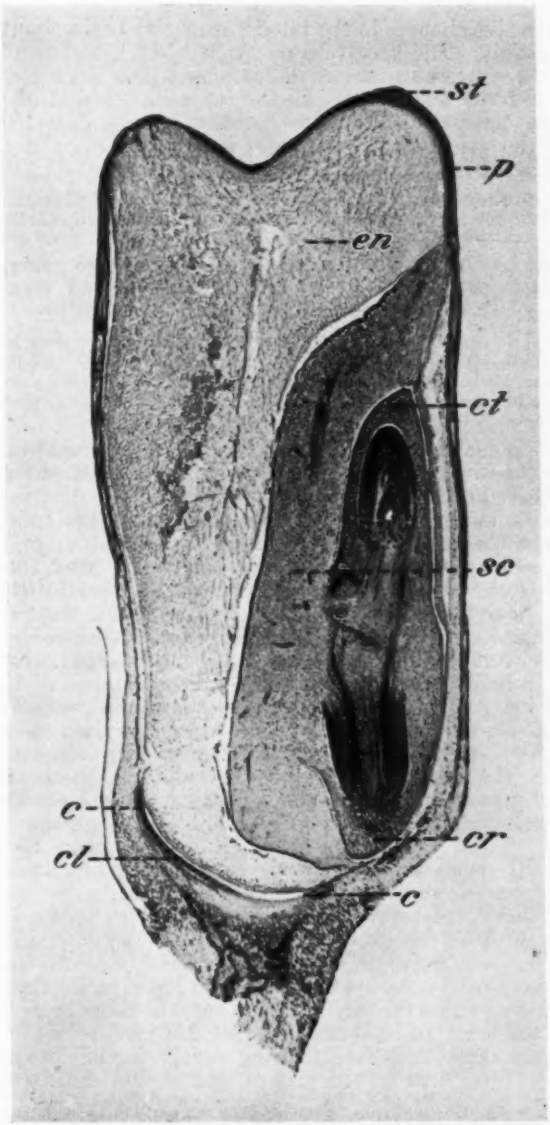


FIGURE 8.—Longitudinal section of a maturing kernel of an L inbred grown at Madison, Wis.; fixed on September 15, 42 days after pollination. In this kernel the embryo appears to be fully formed; the cells of the endosperm are not yet completely filled with starch and the closing layer extends only part way across the hilar orifice (*c-c*). *st*, Remnant of the style; *p*, pericarp; *en*, endosperm; *ct*, coleoptile, *sc*, scutellum; *c*, chalaza; *cr*, coleorhiza; *cl*, closing layer of the hilar orifice. Stained with triple stain. × 14.

Integuments

Degenerative changes in the integuments also begin about the time of fertilization. The literature on the subject has shown a variety of opinions concerning the modifications which take place in the epidermis of the nucellus and in the inner integument of the ovule during the maturation of the caryopsis of corn. True (*10, p. 217*) speaks of the strongly cutinized outer walls of the nucellar epidermis. Concerning the integuments he says:

> Previous to the collapse of the outer integument, the inner integument shows little change. Shortly, however, a slight tendency to weaken is seen, especially in the external layer of cells.

Robbins (*9, p. 173*) lists as a part of the mature grain, "Testa, inner integument of two layers." On the other hand, Randolph (*8, p. 6*) states:

> The absorption of the tissues in question was followed in a close series of stages, and in all the mature kernels studied there was in most cases no nucellar or integument tissue, the aleurone layer of the endosperm lying in close contact with the inner epidermis of the pericarp. In some scattered places a little unabsorbed material was seen, but it never formed a definite layer.

Such statements, indicating that the outer integument in the corn ovary disappears before the inner, as is the case in wheat, or that in the mature kernel there is no layer derived from the integuments, have not been supported by sections examined in the course of this study. On the contrary, certain sections showed more rapid degeneration or resorption of the inner than the outer integument. From other sections it appeared that there had been little, if any, difference in point of time in the disintegration of the two integuments [5] (fig. 7, *A*; fig. 15, *A*). These sections also showed the presence of a thin suberized layer, the semipermeable membrane of the testa, which appeared early in the development of the kernel along the inner surface of the inner integument in contact with the nucellus (fig. 7, *A*). It seems that the suberin was laid down in close contact with the nucellus and inner integument only after degenerative changes in the latter had begun. Attempts to demonstrate the presence of suberin on either of the adjacent walls when the epidermis of the nucellus and the inner integument were separable were unsuccessful. Thus, the origin of the membrane may be open to question. It seems probable, however, that the suberized layer in the corn kernel is derived from the integument, as is the case in wheat (*Triticum vulgare* Vill.)[6] (*7*), bluegrass (*Poa pratensis* L.) (*1*), and Johnson grass (*Sorghum halepense* (L.) Pers.) (*4*), although the number and position of the layers vary with the different genera. In the wheat kernel there are two suberized layers in the testa. They are both laid down on the inner integument, the outer layer becoming much the thicker. In the mature kernel this layer is not of uniform thickness throughout its extent, being visibly thinner in the section over the embryo than in the groove and at the distal end of the

[5] The chances are that Haddad (*3, pp. 9, 11*) also pictures such a condition, although the legend for his figure 4 suggests a different interpretation. From a comparison of his figures 2 and 4 it seems probable that what is considered to be the remains of the outer integument (*fig. 4, 2*) is the inner layer of the ovary wall and that both degenerating integuments, rather than the inner one only, are shown in his figure 4, 3.

[6] According to the rules of botanical nomenclature the name of this species is *Triticum aestivum* L., but as *T. vulgare* is in general use among agronomists and cereal pathologists and geneticists, the writer gives preference to that form.

kernel (7). In the case of *Poa compressa* L. and *P. pratensis* L. the picture is somewhat different. Anderson (*1, p. 1016*) states:

> Soon after fertilization a layer of suberin is found in the cell walls of the inner integument adjacent to the nucellus, and in the cell walls of the outer integument adjacent to the inner integument. Usually the inner suberized layer develops first.

In the mature caryopsis she finds:

> The inner layer of the inner integument consists of a comparatively thin layer of suberin. Its remaining cell walls and their contents are compressed. The inner layer of the outer integument adjacent to the inner integument is composed of a thick layer of suberin. The remaining cells and their contents are presumably dissolved.

In Johnson grass, according to Harrington and Crocker (*4, p. 220*), it is the inner wall of the inner integument that is most highly suberized, although the various layers of the pericarp also contain suberin.

In the corn kernel the membrane is very thin, thinner in some cases than the suberized layer covering the tip of the adjacent coleoptile (fig. 9). It is so thin that variations in thickness that may exist are not easily seen. However, in section it appears to be even more slender for a short distance above the chalaza and over the embryo than elsewhere over the surface of the seed, and slight differences in color intensity when stained with Sudan III suggest the probability that less suberin is present in these areas than at the distal end of the kernel. Treatment of the kernels with iodine-potassium iodide solution also indicates that the membrane is most permeable at the tip of the kernel in the region of the embryo. Furthermore, the parts of the membrane over the embryo and encircling the kernel just above the chalaza seem to be those most easily pierced by fungus hyphae. Kernels carrying a moderately severe diplodia infection show dense masses of hyphae on both sides of the membrane in these regions, while the closed hilar orifice and the distal parts of the kernel may be comparatively free of fungus. Whether the membrane is a thin layer of suberin deposited along the wall of the inner integument, as in the case of the wheat kernel (7), or whether the entire marginal wall itself becomes inpregnated with suberin was not determined. With the exception of this one suberized layer the integuments practically disappear before the kernel is mature.

Nucellus

During the rapid enlargement of the ovary following fertilization the nucellar cells also increase in size. They soon break down and disappear, however, as the rapidly growing endosperm pushes out to occupy the nucellar space (figs. 6, *A*, and 7, *B*, *C*), the epidermis being the last portion of the nucellus to lose its identity (fig. 10, *A*). There seems to be little question that remains of the nucellus are to be found in nearly all kernels, although it is not so clear whether a continuous layer coextensive with the testa, similar to that found in wheat, is the rule. The crushed nucellar cells form a hyaline thread or ribbon in such close contact with the outer walls of the aleurone cells that the two usually appear to be one. Nevertheless, staining reactions occasionally gave a hint of the dual nature of these walls (fig. 10, *B*), while variations in their apparent thickness suggested the same idea.

Infrequently a division of the wall may be observed also. In one kernel from a supposedly mature ear tested on the germinator the sections were torn in such a way that these broad walls split and a

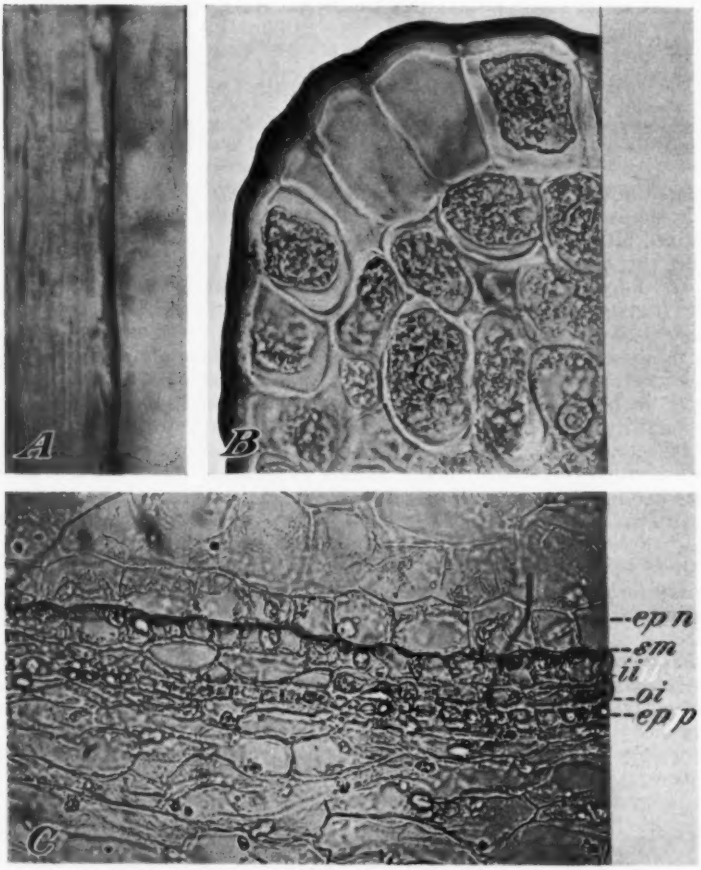


FIGURE 9.—The suberized membrane of the testa. *A* and *B*, Sections from a maturing kernel of inbred Lan grown at Bloomington, Ill., showing the relative thickness of the suberized membrane of the testa over the embryo (*A*) and the suberized layer over the tip of the coleoptile (*B*). Stained with Sudan III, mounted in glycerin. × 535. *C*, Portion of a cross section of a developing kernel of strain A, open-pollinated; fixed on August 19, at which time the silk was beginning to brown. This section is located above the micropyle and shows little of the outer integument. *ep. n*, Epidermis of nucellus; *sm*, suberized membrane; *ii*, inner integument; *oi*, outer integument; *ep. p*, inner epidermis of pericarp. Stained with Sudan III, mounted in glycerin. × 356.

long narrow thread remained adhering to the suberized layer of the testa, although the encircling walls of the aleurone cells were not ruptured (fig. 10, *C*).

Hilum Region

In their study of the caryopsis of Johnson grass and of Sudan grass, Harrington and Crocker (*4*) describe parts that bear a close resemblance to those found in the hilar region in corn. The protective

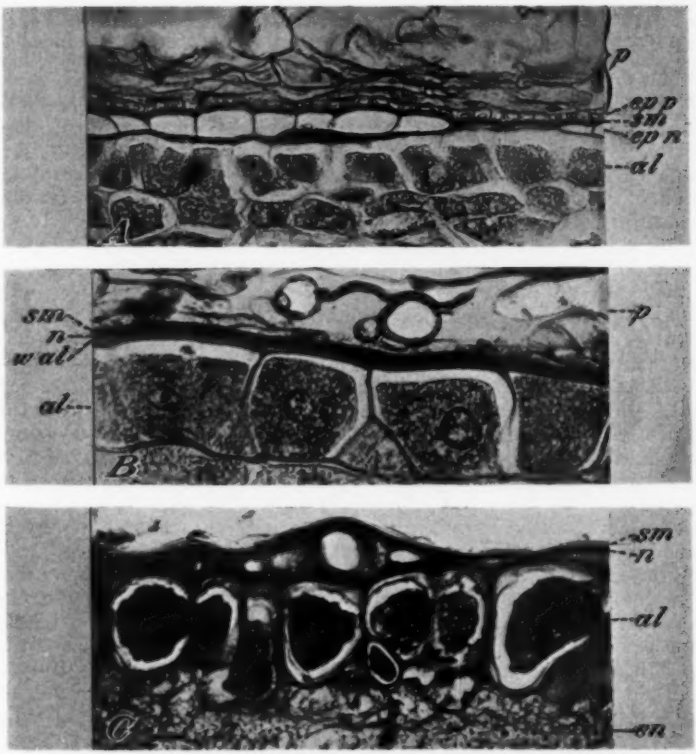


Figure 10.—Sections showing remnants of the nucellus. *A*, Portion of a cross section of a developing kernel of strain A, open-pollinated, grown at Madison, Wis., and fixed on September 6: *p*, Pericarp; *ep. p*, inner epidermis of pericarp; *sm*, suberized membrane of testa; *ep. n*, epidermis of nucellus; *al*, aleurone layer. Stained with triple stain. × 335. *B*, Portion of a longitudinal section of the kernel of inbred Lan shown in figure 9, *A*, *B*. The double nature, in some places, of the broad outer walls of the aleurone cells suggests the presence of remnants of nucellar tissue. *p*, Pericarp; *sm*, suberized membrane of testa; *n*, remnant of nucellar tissue; *w. al*, wall of aleurone cell; *al*, aleurone layer. Stained with Sudan III and gentian violet, mounted in glycerin. × 445. *C*, Portion of a longitudinal section of a dormant, supposedly mature kernel of a Golden Glow strain grown at Madison, Wis., showing what appears to be the remains of the nucellus adhering to the hyaline suberized membrane of the testa: *sm*, Suberized membrane of testa; *n*, nucellar tissue; *al*, aleurone layer; *en*, endosperm. Stained with Delafield's haematoxylin, safranine, and orange G. × 345.

layers are relatively thicker in the grasses than in corn, but otherwise the similarity is striking. They state (*4*, *p. 200*):

> Since in a caryopsis the seed never becomes detached from its pericarp, there is, of course, no true hilum, or seed scar. There is however in the caryopsis of the Andropogonae, a large opening through the inner integument in the position corresponding to the hilum.

They further state (*4, p. 219*):

> The large circular hilar orifice contains no vascular elements, conduction from the vascular bundle of the pedicel and outer layers of the pericarp over the hilar region being by means of parenchymatous pericarp tissue which entirely fills the hilar orifice, and is fused with the inner integument at the hilar margins.
>
> A zone of this conducting pericarp tissue lying just outside the hilar orifice and including the elements which are fused with the inner integument becomes greatly contracted radially and darkly pigmented during the maturation of the caryopsis. This pigmented zone of the pericarp and the inner integument together constitute for the caryopsis an unbroken investment which is extremely resistant to the action of Javelle water and of chromic acid and has the quality of selective permeability, though it probably does not exclude any solute entirely.

As in the above-mentioned grasses, the wide hilar opening through the inner integument in corn is filled with parenchymatous tissue which before fertilization seemed to be of a texture similar to the nucellus (fig. 5, *A*). As the expanded nucellus degenerates, this tissue remains active for a time and with well-marked nuclei in slowly enlarging cells appears by contrast to be densely cellular (fig. 11, *A*).

As the cells attain their maximum size the nuclei disappear (fig. 11, *B*) although the walls continue to take a gentian violet stain with diminishing intensity until, as the kernel matures, they are crushed, contracting radially into a more or less continuous and homogeneous layer which closes the hilar orifice and completes a protective circle of which the semipermeable membrane of the testa is the longer segment. The consistency, continuity, and intensity of color of this compressed layer vary with the age of the kernel and depend somewhat on the strain of corn (figs. 11, *C*; 12, *A*, *B*, *C*). In section, under the microscope, it is yellow to golden brown in color and appears to have a fatty or waxy consistency; to the naked eye, its color is dark brown in the mature grain, and its location and extent may be seen by clipping away the spongy cap of the kernel (fig. 12, *C*). It may be exposed with little difficulty, for, as the moisture content of the maturing kernel is lowered, the tissues of the pedicel forming the so-called "tip cap" of the shelled kernel contract and a cavity forms between the hilar layer and the vascular elements of the cap (fig. 12, *B*). Although this layer is more resistant to hydrolysis by 72-percent H_2SO_4 than adjoining parts, except the semipermeable membrane of the testa, it does not respond to the Sudan III color test for suberin.

Marginal Layer of Endosperm

The cells in the part of the outermost layer of the endosperm that is coextensive with the testa present the appearance usually associated with the aleurone layer in cereals (fig. 13, *A*, *B*). Those at the base of the endosperm facing the hilar region, however, seem to be entirely different. They are more or less completely filled with a streaked substance quite unlike the granular contents of the aleurone cells (fig. 13, *A*, *C*, *D*). Dissimilarity in the nature of the cell contents of the two segments is further indicated in infected kernels. Sections show comparatively few hyphae even making their way intercellularly across the modified layer (fig. 14, *C*), although the aleurone cells are in most cases packed with fungus (fig. 14, *D*). The two sections of the marginal layer of the endosperm show their dissimilarity early; they differ also in thickness, the aleurone layer being for the most part one cell thick, while across the hilum region

marginal modification may extend inward with diminishing intensity to a depth of several cells (fig. 13, *B*, *D*). Until maturity, both are

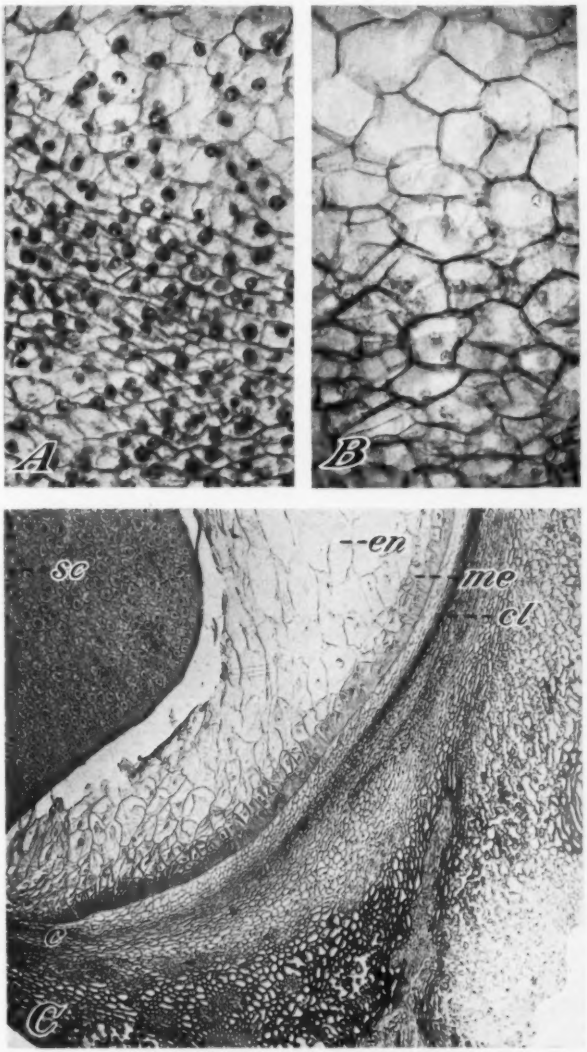
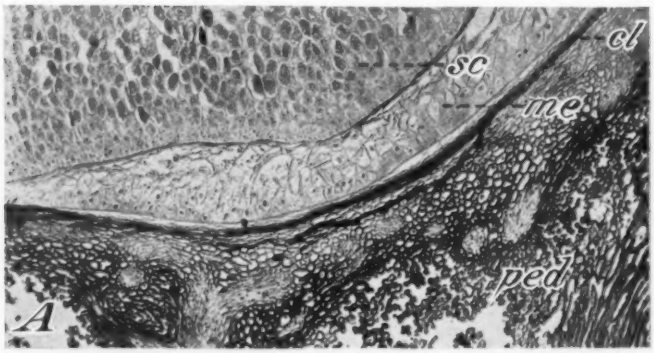
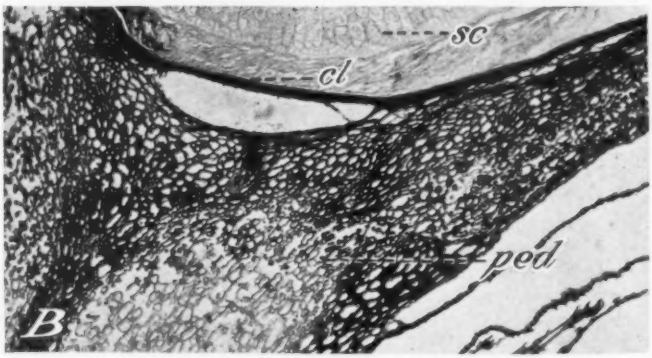


FIGURE 11.—Changes in the cells in hilar region: *A*, Fixed on August 16, 2 days after pollination. × 280. *B*, open-pollinated, fixed on August 19, stained with triple stain. × 280. *C*, portion of a longitudinal section of a maturing kernel of L, fixed 42 days after pollination, showing an early stage in the formation of the closing layer of the hilar orifice; *sc*, scutellum; *en*, endosperm; *me*, marginal layer of endosperm facing the hilar orifice; *cl*, closing layer; *c*, chalaza. Stained with triple stain. × 48.

colorless under the microscope. In the mature kernel parts of the modified marginal layer lying close to the dark layer which bridges





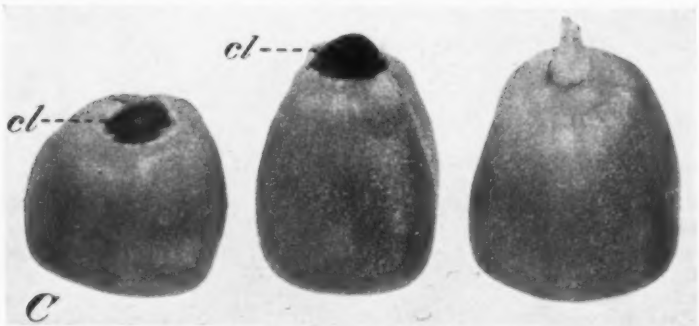


FIGURE 12.—The closing layer of the hilar orifice. *A* and *B*, Portions of longitudinal sections of maturing kernels, showing progressive stages in the formation of the closing layer of the hilar orifice; *cl*, closing layer; *sc*, scutellum; *me*, marginal layer of endosperm facing hilar orifice; *ped*, pedicel. Stained with triple stain. × 48. *C*, Pedicel ("tip cap") removed from two kernels at left to show brown closing layer of hilar orifice (*cl*); at right, kernel with "tip cap." × about 4.

EXPLANATORY LEGEND FOR FIGURE 13

A and *C*, Portions of longitudinal sections through the tip of the kernel in the chalazal region, showing the two parts of the marginal layer of the endosperm. The change in the character of the cells in this layer is abrupt rather than gradual. An arrow points to the line of demarcation in *A*. *A*, From an immature kernel of strain A48 grown at Bloomington, Ill.: *t*, Testa; *p*, pericarp; *al*, aleurone layer; *en*, endosperm; *c*, chalaza; *me*, marginal cells of endosperm facing the hilar orifice; *h*, parenchymatous pericarp tissue in the hilar region. Stained with triple stain. × 98. *B*, Portion of a cross section of a maturing kernel of strain A48 grown at Madison, Wis., showing the cells of the aleurone layer: *al*, Aleurone layer; *se*, starchy

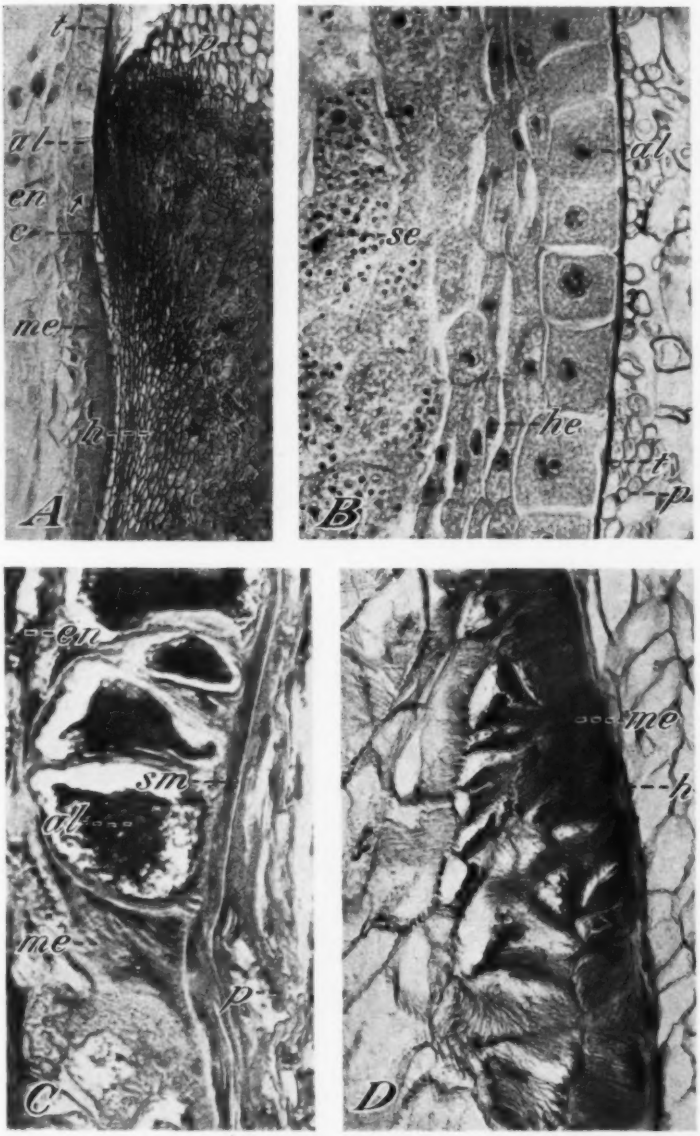


FIGURE 13.—Marginal layer of endosperm. (For beginning of legend see opposite page.)

endosperm; *he*, horny endosperm; *t*, testa; *p*, pericarp. Stained with triple stain. × 285. *C*, From a dormant mature kernel of a Golden Glow strain grown at Madison, Wis. The testa is more highly suberized in this section than in *A* and *B*, as shown by its failure to stain with triple stain. *en*, Endosperm; *sm*, suberized membrane of testa; *al*, aleurone layer; *me*, marginal layer of endosperm opposite the hilar orifice; *p*, pericarp. Stained with triple stain. × 465. *D*, Portion of a section of the marginal layer of the endosperm of a maturing kernel of L corn, showing the character of the cells in the region of the hilar orifice: *me*, Marginal layer of endosperm; *h*, parenchymatous tissue in the hilar orifice. Stained with triple stain. × 285.

the hilar orifice often take on a yellow color. Weatherwax (*12, p. 376*) considers this tissue to have a placental function. He says:

> Its structure and position from the first suggest a special function. Its cells are relatively small, and usually elongated and angular in shape. The nuclei and dense cytoplasm have the appearance of an active living condition, and karyokinetic figures are frequent. It is also in the position nearest the vascular complex which supplies the ovule. These facts indicate that this specialized region has a placental function, taking food from the source of supply and passing it on to other parts of the endosperm. This theory is further supported by the position of this tissue relative to the order of deposition of food in the endosperm.

Pericarp

In the course of development the wall of the ovary becomes the pericarp of the caryopsis. At flowering time the ovary wall consists of a parenchymatous tissue of considerable thickness with a well-defined epidermal layer on the outer surface and a more delicate one on the inner wall.

Some sections show that shortly after degenerative changes appear in the integuments deterioration occurs also in the adjacent layers of the ovary wall (fig. 15, *A*), so that when, a few days after fertilization, these parts have come in contact with one another, it is difficult to identify the limits of each. In other cases, the cells of the inner epidermis are seen to maintain their identity, although they have become more or less separated by the rapid enlargement of the ovule which they had encircled (fig. 10, *A*). As development of the kernel continues, the cells near the base of the ovary and on the inner side of the ovary wall become more spongy in character, while the cell walls of the outer layers thicken, show numerous pits, and respond to safranine stain. Most of the spongy cells of the pericarp, with the exception of those near the base of the kernel, gradually disappear, so that in the mature caryopsis the larger part of the pericarp is composed of thick-walled pitted cells which usually become quite compact over the distal surfaces. Over the coleoptile region of the embryo both types of cells persist in considerable numbers, while in the area to be ruptured by the emerging coleorhiza only the thin-walled parenchyma is found (fig. 15, *B*). A relatively thin layer of cutin covers the outer surface of the pericarp.

Beeskow [7] has reported finding traces of suberin in the pericarp of a strain of Hickory King corn with which he was working. Its presence in any considerable quantity in the pericarp, tip cap, or rachilla might possibly be an important factor contributing to resistance to fungus invasion. In the sections examined by the writer, however, no barriers of this nature were demonstrated in these regions, although the presence of the thin suberized membrane of the testa was clearly shown.

When the hull is peeled from a soaked kernel, cleavage is along the line of least resistance. In the mature corn kernel this is in the spongy inner layer of the pericarp, outside the circle of the semipermeable membrane of the testa and the closing layer of the hilar orifice (fig. 10). The removable hull thus consists of pericarp tissue and does not include the suberized semipermeable membrane of the testa.

[7] Beeskow, H. C. The selective semipermeability of the seed coat of corn. (Unpublished master's thesis, Univ. Chicago.) 1924.

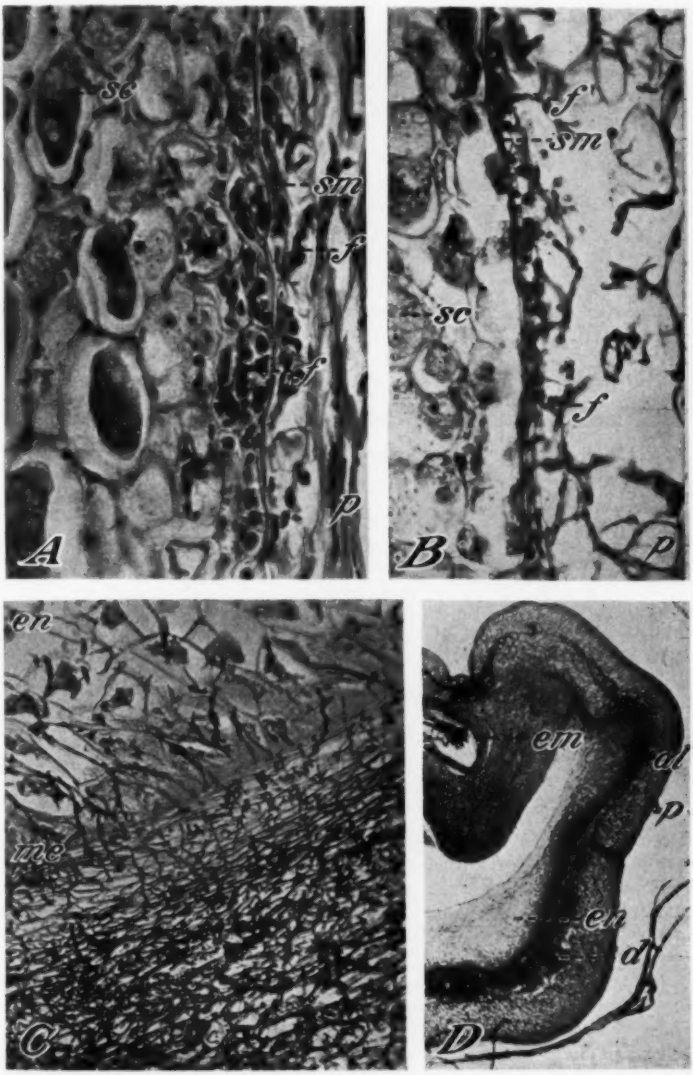


FIGURE 14.—Sections from kernels infected with *Diplodia*. *A* and *B*, Longitudinal sections, showing portions of the suberized membrane of the testa over the embryo. The membrane exhibits considerable resistance to fungal penetration and preserves its identity after the walls of neighboring infected cells have almost disappeared. *sc*, Scutellum; *sm*, suberized membrane; *f*, fungus hyphae; *p*, pericarp. Stained with Delafield's haematoxylin, safranine, and orange G. × 430. *C*, Portion of a longitudinal section through the hilum region of an infected immature kernel. The closing layer has not been formed and hyphae have advanced from the pedicel into the endosperm. *en*, Endosperm; *me*, marginal layer of endosperm facing the hilar orifice. Stained with Delafield's haematoxylin, safranine, and orange G. × 160. *D*, Part of a cross section of a badly infected kernel. Hyphae have invaded all tissues and are massed in the aleurone cells. *al*, Aleurone layer; *em*, embryo; *p*, pericarp; *en*, endosperm; *d*, *Diplodia* pycnidia. × 17.

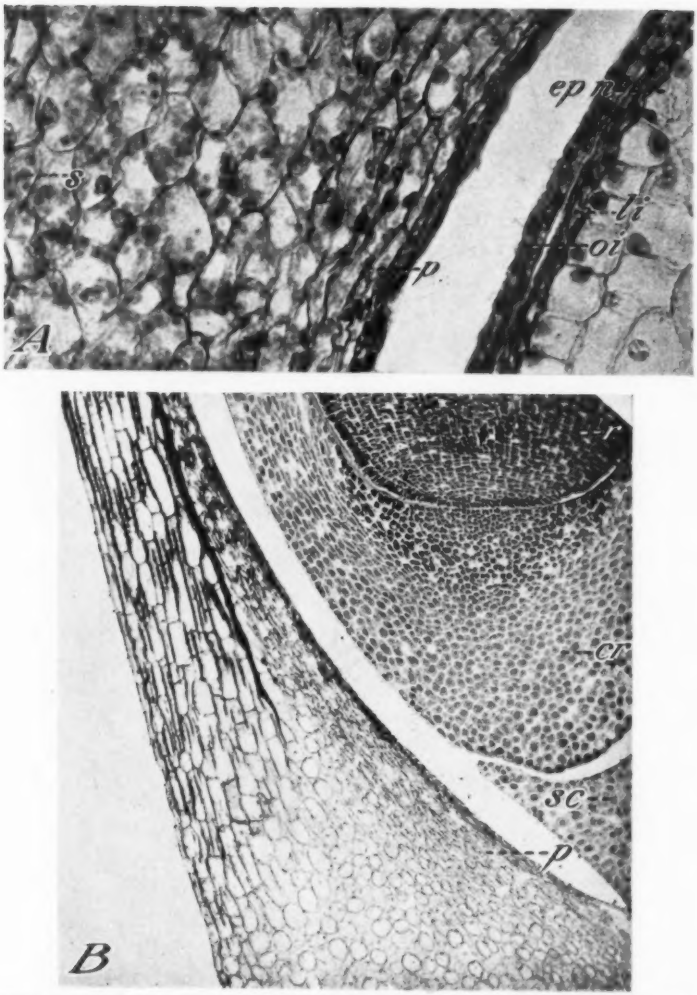


FIGURE 15.—Sections of the pericarp at different stages of development. *A*, Portion of a longitudinal section of a developing kernel fixed shortly after fertilization, showing degenerative changes beginning in the inner layers of the pericarp and integuments. There is an abundance of starch in the cells of the pericarp. *ep. n*, Epidermis of nucellus; *ii*, inner integument; *oi*, outer integument; *p*, pericarp; *s*, starch. Stained with triple stain. × 430. *B*, Portion of a longitudinal section of a maturing kernel, showing the spongy structure of the pericarp in the region to be ruptured during germination by the emerging coleorhiza: *r*, Root tip; *cr*, coleorhiza; *sc*, scutellum; *p*, pericarp. Stained with triple stain. × 58.

DISCUSSION

Examination of the varied and representative material from pedigreed strains of yellow dent corn grown at Bloomington, Ill., and Madison, Wis., provided by collection of specimens at intervals during the summers of 1930–33 failed to show the presence of anatomical differences sufficient in themselves to account for high degrees of resistance or susceptibility to diplodia kernel rot manifested in the field (figs. 16, 17). However, variations which may have a bearing on the problem were observed (1) in the speed with which the hilar orifice is closed, (2) in the thickness and compactness of the closing layer, and (3) in the completeness of the union of the closing layer with the ends of the suberized membrane of the testa. The brown closing layer of the hilar orifice is, at its best, relatively thick and impervious to fungi and when joined to the ends of the suberized semipermeable membrane of the testa blocks advance into the seed in that region. If, however, formation of the hilar layer is delayed or incomplete, hyphae may pass around the ends of the testa and enter the embryo by way of the hilar orifice (fig. 18, *A*, *B*, *C*). From the material examined it may be said in general that it was in those strains known to be most susceptible in the field that a delayed and less effective closing of the hilar orifice was found. Not only is the presence of an effective closing layer in the hilar orifice an actual barrier to fungus advance through that particular region of the kernel, but it seems possible also that it should be considered as associated with certain phases of the maturation process of the caryopsis. In that case, the early appearance of the closing layer, in addition to imposing an efficient physical barrier to hyphal advance, may also be indicative of chemical changes which afford a less favorable nutrient medium for fungus growth within the plant.

In cereals the suberized semipermeable membrane of the testa functions as a more or less effective barrier to fungus advance. In the case of wheat the three-layered testa is considered to be the part of the kernel most resistant to penetration by *Gibberella saubinetii* (Mont.) Sacc., becoming increasingly resistant as the kernel matures (*7*). The outer suberized layer is the most resistant part of the testa. It varies in thickness and its resistance is seemingly proportional to its thickness. In section, it is sufficiently broad to show distinct differences in width in the embryo and distal regions of the kernel. In the corn kernel, however, the single suberized layer of the testa is so thin that any variations in thickness which may exist are not easily seen. Nevertheless it seems probable that it also is thinnest over the embryo and in the proximal region of the kernel, for it is in these areas that its penetration by fungus occurs most frequently. Though it appears so delicate, the membrane is relatively resistant to hyphal penetration and maintains its identity surprisingly well despite the fact that no instances were observed in which hyphae massed in the adjacent pericarp failed to pierce it (fig. 14, *A*, *B*). Once within the circle of the testa, fungus growth in the aleurone layer is rapid. These cells seemingly present a very favorable medium for *Diplodia* and soon become packed with hyphae. It is in this region also that most of the pycnidia in the kernel may be found (figs. 14, *C*; 19).

FIGURE 16.—Ears of cross 58, a strain of yellow dent corn that has proved resistant to diplodia ear rot in the field. Inoculated by a hypodermic injection of a spore suspension of *Diplodia zeae* on August 25; harvested September 12 at Bloomington, Ill. *A*, Tip inoculated; *B*, shank inoculated.

FIGURE 17.—Ears of Krug × Lan, a strain of yellow dent corn that has proved susceptible to diplodia ear rot in the field. Inoculated hypodermically with a spore suspension of *Diplodia zeae* on August 25 and harvested September 12 at Bloomington, Ill. *A*, Shank inoculated; *B*, tip inoculated.

During the development of the kernel, changes in the carbohydrate content of the endosperm occur which may also have a bearing on the host-fungus relationship in that region. In her microchemical studies of the developing endosperm of maize, Lampe (*5*) reports finding

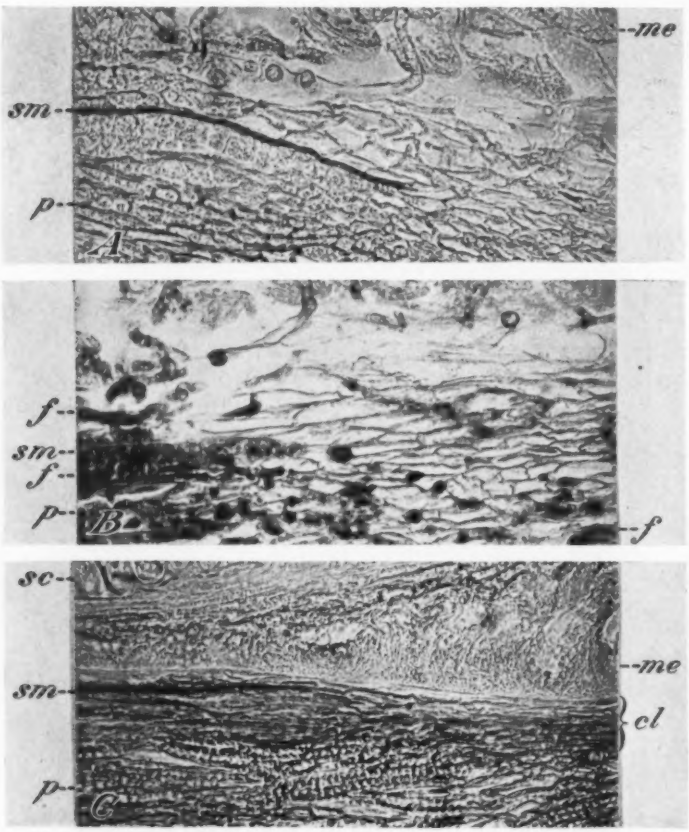


FIGURE 18.—Detail sections of kernels from ears shown in figures 16 and 17. *A*, Portion of a longitudinal section of an infected kernel of the Krug × Lan strain harvested September 12 (fig. 17), showing the end of the suberized membrane of the testa and the absence of the closing layer in the hilar orifice: *me*, Marginal layer of endosperm facing the hilar orifice; *sm*, suberized membrane of testa; *p*, pericarp. Stained with Sudan III, mounted in glycerin. × 335. *B*, Section from same kernel as *A*, stained with Delafield's haematoxylin, safranine, and orange G to show the presence of hyphae in the hilar orifice and on both sides of the suberized membrane: *f*, Fungus hyphae; *sm*, suberized membrane; *p*, pericarp. × 335. *C*, Portion of a longitudinal section of a kernel of cross 58 harvested on September 12 (fig. 16), showing the presence of the closing layer of the hilar orifice and its close contact with the end of the suberized membrane of the testa. The contrast of the natural yellow color of the closing layer with the hyaline cells on either side is lost in the photomicrograph. *sc*, Scutellum; *sm*, suberized membrane; *p*, pericarp; *me*, marginal layer of endosperm; *cl*, closing layer. Stained with Sudan III, mounted in glycerin. × 335.

reducing sugars present during the early development in all types of corn. In older kernels polysaccharides were found in the distal part and reducing sugars at the base. As the region containing polysaccharides increased, that containing reducing sugars decreased, until

at maturity, in nonsweet corn, the disappearance of sugars was essentially complete.

Infection in the kernel appears usually to occur at the proximal end of the caryopsis. Hyphae were observed to enter the pedicel near the bases of the glumes or to have entered from the cob. This accords with Branstetter's (*2, p. 17*) findings as to the location of fungi in infected kernels shown by his platings. He states:

> These results seem to warrant the assumption that if a corn grain is infected, the tip one-fifth invariably contains the fungus, since in no case when the tip failed to show infection did any other part of the kernel show infection.

FIGURE 19.—Detail of figure 14, *D*. Pycnidia of *Diplodia zeae*, containing mature spores, located along the aleurone layer in a badly infected kernel. Stained with Delafield's haematoxylin, safranine, and orange G. × 220.

In a large percentage of diplodia inoculations in the field under conditions favorable for infection, hyphae were found in the spongy tissues of the pedicel and the proximal pericarp of the kernel. In some cases these regions supported an abundant fungus growth; in other strains a few straggling hyphae were visible. Since no anatomical differences sufficient to account for the two conditions were apparent in the hosts, it seems not unreasonable to suppose that such lack of uniformity in the host-fungus relationship may perhaps be tied up more closely with the chemical medium offered by the host in its relation to the nutritional requirements of the parasite than with anatomical differences in kernel structure. Further experiments along this line have been undertaken.

SUMMARY

This paper outlines briefly the development and microscopic anatomy of the caryopsis of dent corn and is concerned primarily with those structures which might have a bearing on the problem of host resistance and susceptibility to fungus diseases in general and to invasion by *Diplodia zeae* (Schw.) Lév. in particular.

Unless otherwise noted, the specimens examined were from hand-pollinated ears of yellow dent corn grown at Bloomington, Ill., and Madison, Wis. During the early periods of development of the kernel, collections were made at intervals varying from 2 to 7 days.

Sections of the ovary showed that the outer integument covers all but a relatively narrow triangle of the surface of the inner integument and that the outer and inner integuments degenerate at about the same time, leaving a testa consisting of a single very thin suberized semipermeable membrane, which originated along the inner wall of the inner integument in close contact with the epidermis of the nucellus. It was not determined whether the entire marginal wall of the integument had been impregnated with suberin or whether a layer of suberin had been laid down along its surface as is the case in the wheat kernel. The presence of the membrane was not demonstrated as long as the inner integument and the epidermis of the nucellus were separable.

The two sections of the marginal layer of the endosperm are pictured. The formation of the closing layer of the hilar orifice is shown, and attention is called to the possible relation of the variations found in this region to fungus advance into the embryo. Although these points may have a bearing on the problem of resistance or susceptibility to kernel rots, no anatomical features were observed sufficient in themselves to account for the differences in resistance or susceptibility shown by various strains of corn in the field.

Diplodia infection in the kernel was found to begin in almost every case at the proximal end of the kernel. In some strains the spongy tissues of the pedicel and proximal pericarp were filled with the fungus; in others, only a few straggling hyphae could be found in those regions. Anatomical features sufficient in themselves to account for such differences did not appear to be present. It seems, therefore, not unreasonable to conclude that such lack of uniformity in the host-fungus relationship may perhaps be tied up more closely with the chemical medium offered by the host in its relation to the nutritional requirements of the parasite than with anatomical differences in kernel structure.

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INFLUENCE OF WEATHER FACTORS ON MOISTURE CONTENT OF LIGHT FUELS IN FORESTS OF THE NORTHERN ROCKY MOUNTAINS[1]

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INTRODUCTION

The necessity of forest-fire protection is generally recognized in the United States. The tremendous damage done by forest fires each year to valuable timber, watershed cover, forest range, wildlife, recreational facilities, and personal property has impressed upon the people the need for preventing and controlling forest fires so far as this is humanly possible.

In the forests of northern Idaho and of Montana the fire-protection problem is particularly difficult, owing to the inflammable character of the forest vegetation and to the dryness of the summer weather. On the 23 million acres of national-forest land in this area, during the 10-year period 1925–34 there occurred a yearly average of 1,356 fires, which burned over 114,000 acres, caused damage totaling nearly three-quarters of a million dollars, and required expenditure of a similar amount for control.

Fire-control experience has shown that in this northern Rocky Mountain region approximately 50 percent of the area burned and 35 percent of the suppression costs result from only 1 percent of the fires.

Obviously, in this region satisfactory fire control can be attained only if the fire-protection organization succeeds in suppressing fires while they are still small. In efforts toward this end, administrative officers are aided by knowledge of current forest-fire danger.[3] A complete understanding of fire danger from day to day is difficult to obtain because many factors are involved, including season of year, activity of fire-starting agencies, topography, character of green vegetation, weather, fuel type, fuel volume, and fuel moisture content. By making observations or measurements of several of the most important factors and properly integrating them, however, the forest protectionist can determine the relative fire danger existing at a given time and place. In this way he can learn whether his force should be temporarily expanded or reduced, and how it should be distributed over the area for which he is responsible. In addition, through a knowledge of the influence of given factors on fire danger the protectionist is assisted in interpreting weather forecasts and thus in preparing for all classes of fire danger that are likely to arise.

[1] Received for publication July 29, 1935; issued February 1936.

[2] The writer is indebted to F. X. Schumacher, of the Division of Silvical Research, Forest Service, for guidance in planning the statistical analysis involved in this study.

[3] "Forest fire danger" as used here is a general term expressing the sum total of the factors that determine whether fires will start, spread, and do damage.

SIGNIFICANCE OF MOISTURE CONTENT OF FOREST FUELS

Fuel-moisture content alone serves as a criterion of forest-fire danger. The wetness or dryness of forest fuels largely determines their inflammability or ease of ignition and the rate of spread of forest fire. Obviously, each fuel must be heated to a certain temperature before combustion results, and anything that retards this heating reduces fire danger. Water in the fuels absorbs heat before it is driven off in the form of vapor; consequently it delays the raising of fuel temperatures to the kindling point. Knowledge of the relation of the moisture content of the common forest fuels to their inflammability, or to the rate of spread of fire, or to both, is essential in determining current fire danger.

The moisture content of lightweight fuels such as duff and small branch wood is particularly important because of their wide-spread distribution in almost all forest types and because they serve as carriers of fire from tree to tree and from log to log. Duff commonly composes a greater part of the surface layer of the forest floor than any other single fuel.

Show (*9*),[4] Larsen (*7*), Stickel (*10, 11*), and Gisborne (*3*) proved the important relation of moisture content to the inflammability of duff [5] by making burning tests. The latter two workers tested in detail the ease of igniting duff of given moisture content with different firebrands. Gisborne, working with undisturbed duff in western white pine (*Pinus monticola*) forests in northern Idaho, concluded that whenever the duff has less than 10 percent moisture content it can be ignited by any firebrand producing heat equal to that of an ordinary match, that at more than 13 percent moisture the duff is generally immune to ignition by burning matches, and that camp fires can ignite the duff and cause fire to spread through it whenever it contains less than 18 percent moisture. On the basis of these findings and of general observation, Gisborne defined the following six degrees of susceptibility of the top layer of duff to ignition by various common firebrands:

Degree of susceptibility:	*Moisture content (percent)*
Noninflammable	More than 25.
Very low inflammability	25 to 19.
Low inflammability	18 to 14.
Medium inflammability	13 to 11.
High inflammability	10 to 8.
Extreme inflammability	7 to 0.

Stickel (*10*) made a detailed investigation in the Adirondack Mountains to determine the moisture content necessary to prevent ignition of duff by matches, cigarette butts, pipe heels, locomotive sparks, and small camp fires. His results, from 370 tests, are summarized in table 1.

[4] Reference is made by number (italic) to Literature Cited, p. 905.

[5] In this discussion the term "duff" applies to the surface layer of the forest floor, that is, to the "litter", which is made up of vegetable-matter deposits only slightly decomposed.

TABLE 1.—*Surface duff-moisture contents at which various firebrands are effective* [1]

Degree of hazard [2]	Surface duff moisture content, percent	Firebrands effective
Extreme	Less than 6	Cigarettes, locomotive sparks, pipe heels, matches, and camp fires.
High	6 to 10	Locomotive sparks, pipe heels, matches, and camp fires.
Medium	11 to 16	Pipe heels, matches, and camp fires.
Low	17 to 22	Matches and camp fires.
Very low	23 to 29	Camp fires.[3]
Generally safe	30 or more	None.

[1] According to findings of Stickel (*10*).
[2] "Hazard", in Stickel's classification, has the same meaning as is attached in this paper to "forest-fire danger" (see footnote 3, p. 885[1]).
[3] Duff at edge will smolder, but fire will not spread much.

Rate of spread of forest fire has definitely been shown to depend principally upon fuel-moisture content. The Quartz Creek fire of 1926 (*2*) and the Freeman Lake fire of 1931 (*4*), both in northern Idaho, made their longest runs on days when duff moisture was exceptionally low. The latter fire, which spread at an average rate of 1,600 acres per hour for 12½ hours, began at a time when the duff-moisture content was about 5 percent. In no recorded instance has a "blow-up" occurred when the moisture content of lightweight fuels was relatively high.

DETERMINANTS OF FUEL MOISTURE CONTENT

The several weather elements, acting together, are the most important determinants of forest fuel moisture content. When the weather is wet, the fuels are wet or tend to become so; and dry weather produces dry conditions in the fuels. The changes, of course, require more or less time, the lag depending upon how wet or dry the fuels were to begin with. In the northern Rocky Mountains, after a heavy rain a week or more of hot, drying weather may be required to produce extreme danger.

The moisture content of light fuels such as duff and small branch wood bears an especially close relation to current weather. Duff, in particular, because of its loose distribution and porous structure, picks up or loses moisture rapidly when atmospheric conditions change.

Differences in the moisture contents of similar fuels on parts of the same area may be due in part to weather variations between one place and another, but no doubt are due principally to differences in degree of exposure of the fuels. A dense forest canopy intercepts most of the direct rays of the sun and reduces wind movement, thereby lowering temperature and evaporation and increasing humidity beneath it (*5*). Fuel moistures, consequently, are higher under dense canopies than they are on burned-over or clear-cut areas fully exposed to sun and wind.

METHODS AND CONDITIONS OF FIELD STUDY

The study discussed here has dealt for the most part with duff-moisture content in its relation to current weather. It has dealt also with moisture content of branch wood one-half inch in diameter in its relation to weather. These relations were observed under different forest-canopy conditions.

In this study duff-moisture measurements have been confined to the uppermost ½-inch layer, because when the moisture content of this layer is low fire may spread through it rapidly even if the average moisture content of the entire forest floor is high.

Measurements of weather factors and fuel moisture have been made on three exposures at the Priest River branch of the Northern Rocky Mountain Forest and Range Experiment Station, in northern Idaho. Beginning in 1924, observations were taken on an area from which all trees had been removed; on an area where timber cutting had reduced the forest canopy by about half, the principal tree species of the remaining stand being western hemlock (*Tsuga heterophylla*); and in a dense stand of western white pine near the base of

FIGURE 1.—Clear-cut station, fully exposed to weather.

a northwest slope. These areas were approximately three-quarters of a mile apart. In 1930 the original half-cut and full-timbered stations were abandoned, and new ones established on a bench within 1,400 feet of the clear-cut station. On the new half-cut station, the residual stand is similar in composition to that on the one abandoned. The new full-timbered station is occupied by the climax western white pine type, in which western hemlock and western red cedar (*Thuja plicata*) predominate. Comparative light values taken at the instrument exposures by Haig [6] are shown in table 2. The clear-cut station, and the half-cut and full-timbered stations in use from 1930, are shown in figures 1, 2, and 3, respectively.

[6] HAIG, I. T. CERTAIN FACTORS CONTROLLING INITIAL SEEDLING ESTABLISHMENT IN WESTERN WHITE PINE STANDS. 1935. (Unpublished doctor's dissertation. Copies on file at Yale Univ. School of Forestry, New Haven, Conn., and Northern Rocky Mountain Forest and Range Expt. Sta., Missoula, Mont.)

FIGURE 2.—Half-cut station; residual stand is composed chiefly of western hemlock.

FIGURE 3.—Full-timbered station; the dense virgin stand is composed chiefly of western hemlock and western red cedar.

TABLE 2.—*Light values on half-cut and full-timbered areas in use from 1930, as determined with different instruments*

Instrument	Light, in terms of full sunlight, on—	
	Half-cut area	Full-timbered area
	Percent	*Percent*
Clements photometer	24	4
Shirley thermopile	23	3
Livingston black and white spheres	26	5
Average	24.3	4

Careful study revealed that the old and new stations were nearly enough alike to justify joint analysis of records obtained before and after 1930.

Simultaneous measurements of weather factors and duff-moisture content on the clear-cut, half-cut, and full-timbered areas were begun in June 1924. They were made daily at 4:30 p. m. Air temperature, relative humidity, precipitation, evaporation, and temperature of the dew point were the weather elements measured in 1924. Other elements were added later. Table 3 lists the factors investigated and shows in what years measurement of each factor was begun on the three areas, respectively. Beginning with 1925, twice-daily measurements were made at all stations throughout the fire season, at approximately 9 a. m. and 4:30 p. m. These two hours were selected in order to sample extremes, preliminary investigation having indicated that fuel moisture normally was greatest between 7 and 9 a. m. and least at about 4:30 p. m. The dates on which measurements were begun and ended each year were not fixed. In general, measurements were begun in April or May, before the fire season actually started, and were terminated in late September or early October after the fire season definitely came to an end.

TABLE 3.—*Fuel-moisture and weather factors studied, and years in which measurement of each was begun on the three study areas, respectively*

Factor	Year in which measurements were begun on—		
	Clear-cut area	Half-cut area	Full-timbered area
Fuel moisture content:			
Duff moisture	1924	1924	1924
½-inch wood moisture	1929	1929	1929
Weather elements and associated factors:			
Maximum air temperature	1924	1930	1930
Current air temperature	1924	1924	1924
Minimum air temperature	1924	1930	1930
Maximum duff temperature	1930	1930	1930
Temperature of the dew point	1924	1924	1924
Current relative humidity	1924	1924	1924
Minimum relative humidity	1924	1930	1930
Average relative humidity	1924	1930	1930
Wind	1930	1930	1930
Precipitation	1924	1930	1930
Evaporation	1924		
Number of days since 0.01 inch of precipitation	1924	1924	1924
Number of days since .10 inch of precipitation	1924	1924	1924
Number of days since .20 inch of precipitation	1924	1924	1924
Number of days since .30 inch of precipitation	1924	1924	1924
Number of days since .40 inch of precipitation	1924	1924	1924

ANALYSIS OF DATA, AND APPLICATION OF RESULTS OF ANALYSIS

Gross correlations indicate the forest-fuel moistures that normally exist at given air temperatures and humidities; they fail, however, to show the relative or net influence of temperature or of humidity on fuel-moisture content. None of the weather elements acts independently of the others. Hence, in order to determine fundamental relations between fuel-moisture content and the weather it was necessary to subject weather and fuel-moisture data to multiple-correlation analysis.

METHOD OF ELIMINATING CURVILINEARITY

A preliminary multiple-correlation study involving duff moisture as the dependent variable and several weather elements as the independent variables produced, in all cases, curvilinear regressions.

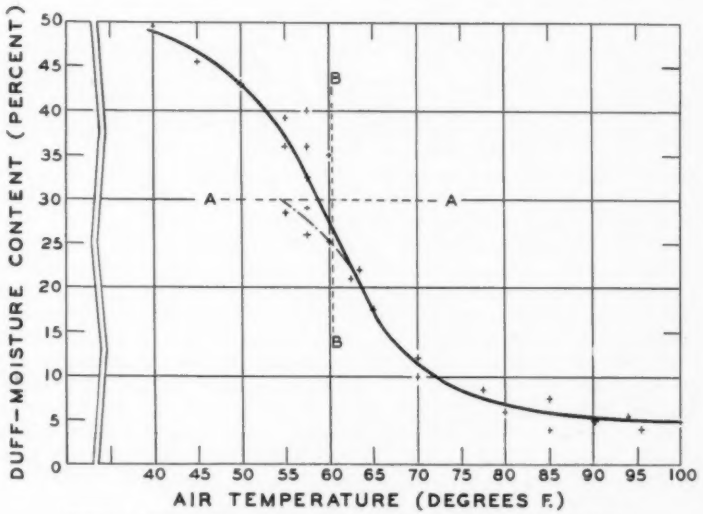

FIGURE 4.—Hypothetical regression of duff-moisture content on air temperature.

In multiple-correlation problems it is desirable to obtain algebraic expression of regressions whenever possible, because they are the simplest to compare and interpret and are not susceptible to the personal error associated with fitting freehand curves. The curvilinearity of these regressions was due to the fact that the instrument used to measure duff moistures does not show values greater than 50 percent. Direct elimination of observations of duff-moisture content amounting to, say, 30 percent or more inevitably lessens the average slope of the regression curve. These data can be eliminated without altering the slope, however, by placing a limit on an independent variable. An illustration of the effects of these two procedures follows: In figure 4 if all observations of duff moisture greater than 30 percent—that is, all the points above line *A*—are directly eliminated, the upper end of the line of best fit is changed from its former position to the one shown by the dash-dot line. Lim-

itation of the dependent variable, then, has changed the slope of the regression curve. However, if all observations made when air temperature was 60° F. or lower—that is, all points to the left of line *B*—are rejected all duff moistures of 30 percent or more are eliminated from the correlation without causing a change in the slope of the regression curve.

If study is restricted to observations made on the clear-cut area 2 or more days after 0.01 inch of precipitation, and on the half-cut and full-timbered areas 5 or more days and 6 or more days, respectively, after 0.01 inch of precipitation, all duff moistures of 30 percent or more are eliminated. This removes the curvilinearity in the net regressions due to restriction of the dependent variable to 50 percent. It is logical to confine the study to duff moistures of less than 30 percent, because when duff contains 30 percent or more moisture fire danger does not exist.

SELECTION OF MOST IMPORTANT VARIABLES

As is shown in table 3, data on 16 weather elements were available for analysis. This large number of variables cannot be handled conveniently by the usual correlation methods. It was thought possible that certain of these factors contribute nothing of significance to the final multiple-correlation coefficient, although each shows a definite relation to fuel moisture when correlated with it separately. For these reasons, measures were taken to select those weather elements that contributed most to the multiple correlation.

Analysis of a random sample of 130 sets of afternoon measurements for the clear-cut area according to the method developed by Kincer and Mattice (*6*) showed that the factors having the greatest effect on fuel moisture were current air temperature and relative humidity. Maximum duff temperature and evaporation rate were correlated almost as closely with fuel moisture as current air temperature and relative humidity, respectively. Factors having comparatively small influence on fuel moisture were temperature of the dew point, number of days since 0.01 or 0.20 inch of precipitation, and wind. These variables were retained for further investigation in preference to mean and minimum relative humidity, for example, because the latter are highly correlated with current relative humidity.

On the basis of all the available afternoon records for the clear-cut area that were made more than a day after 0.01 inch of precipitation, gross-correlation coefficients were computed for duff moisture as the dependent variable with each of the following eight factors as independent variables: Current relative humidity, current air temperature, maximum duff temperature, evaporation, temperature of the dew point, number of days since 0.01 inch of precipitation, wind, and number of days since 0.20 inch of precipitation. The results are given in table 4. Because wind and number of days since 0.20 inch of precipitation were not found to be significantly correlated with either the dependent variable, duff moisture, or with any of the other variables tested, they were dropped from consideration.

TABLE 4.—*Gross-correlation coefficients*[1] *for all combinations of duff-moisture content (X) on clear-cut area and eight weather factors*

Correlation coefficient r	Numerical value	Correlation coefficient r	Numerical value	Correlation coefficient r	Numerical value	Correlation coefficient r	Numerical value
XA	+0.660	AD	−0.608	BH	+0.103	EF	−0.227
XB	−.576	AE	+.597	CD	+.734	EG	−.169
XC	−.552	AF	+.229	CE	−.053	EH	−.049
XD	−.535	AG	−.051	CF	+.064	FG	−.011
XE	+.312	AH	−.058	CG	−.010	FH	+.161
XF	−.146	BC	+.784	CH	+.001	GH	−.093
XG	−.037	BD	+.740	DE	−.007		
XH	−.028	BE	−.020	DF	+.035		
AB	−.727	BF	+.133	DG	+.088		
AC	−.597	BG	−.121	DH	−.193		

[1] Based on 226 sets of afternoon observations taken 1 or more days after 0.01 inch of precipitation. Explanation of symbols: A=relative humidity; B=air temperature; C=maximum duff temperature; D=evaporation; E=temperature of the dew point; F=number of days since 0.01 inch of precipitation; G=wind; H=number of days since 0.20 inch of precipitation.

JOINT CORRELATION

When a multiple correlation of duff moisture with air temperature and relative humidity was made, a joint relation was evident. A joint relation of variables is the relation existing when the change in a dependent variable that corresponds with a change in one independent variable depends in part upon the magnitude of a second

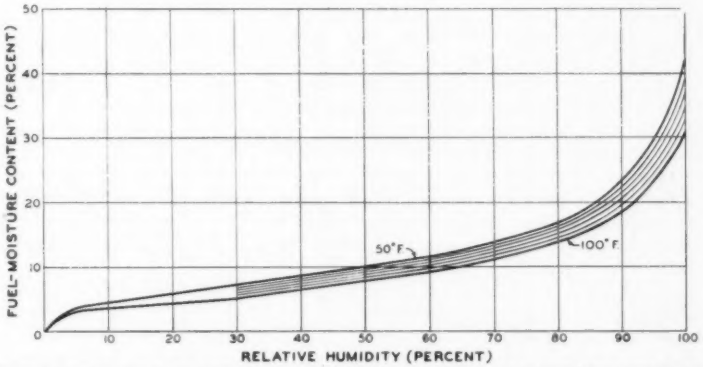

FIGURE 5.—Curve of moisture contents of coniferous duff and branch wood in equilibrium with air temperature and humidity (as determined by M. A. Dunlap).

independent variable. The equilibrium relations between fuel moisture and relative humidity at different air temperatures determined by Dunlap,[7] of the Forest Products Laboratory, and shown in figure 5 also indicate a joint relation.

The standard linear-regression equation of the form

$$DM=aT+bRH+K \qquad (1)$$

in which

DM=Duff-moisture content
T=Air temperature
RH=Relative humidity

and

a, b, K=Constants

[7] DUNLAP, M. A. THE RELATION OF HUMIDITY TO THE MOISTURE CONTENT OF FOREST FIRE FUELS. 1924. [Unpublished manuscript. U. S. Dept. Agr., Forest Prod. Lab., Madison, Wis.]

does not show this relation. This type of equation results in parallel regression lines that do not fit the data except when all three variables are at their mean values. In other words, it represents the change in duff moisture caused by a change in relative humidity as constant regardless of air temperature. In order to reveal the true relation of air temperature and relative humidity to duff moisture, joint-correlation methods must be used.

A regression equation of the type

$$DM=a\left(\frac{RH}{T}\right)+K \qquad (2)$$

was found to fit the data, as is clearly illustrated in figures 6 and 7. The lines of regression in figure 6 represent values computed separately

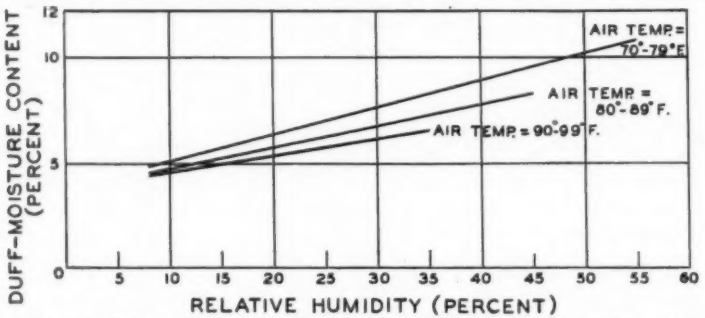

FIGURE 6.—Average actual moisture content of duff on the clear-cut area at given relative humidities and air temperatures. (Basis, 206 sets of observations taken at 4:30 p. m.)

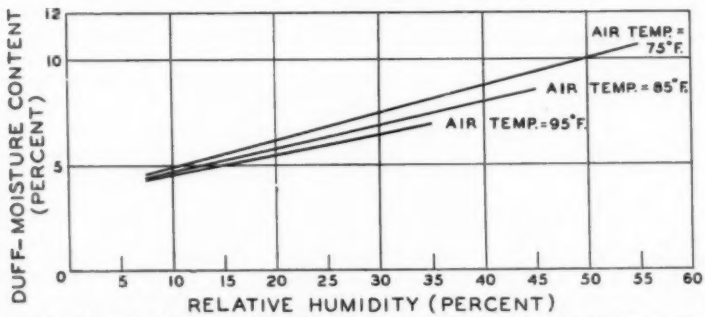

FIGURE 7.—Average moisture content of duff on the clear-cut area at given relative humidities and air temperatures, as estimated by use of the regression equation $DM=9.64\left(\frac{RH}{T}\right)+3.55$. (Basis, 226 observations taken at 4:30 p. m.)

for each of the temperature classes shown; those in figure 7 were located by substituting values of temperature and humidity in equation 2.

The use of the term $\frac{RH}{T}$ is explained as follows:

Relative humidity is the ratio between the quantity of water vapor in a unit of space and the maximum quantity that unit of space can

hold, temperature and pressure remaining constant. Absolute humidity is the actual quantity of moisture in a unit of space, usually expressed in grains per cubic foot. Let

AH = Absolute humidity
AH_s = Absolute humidity at saturation.

Then, by definition $RH=\frac{AH}{AH_s}$ when temperature and pressure are constant.

But AH_s depends entirely on T. Then $RH=\frac{AH}{f(T)}$, where $f(T)=$ some function of T, or $f(T)$ $(RH)=AH$. If $f(T)=\frac{1}{T}$, then $\frac{RH}{T}=AH$. In other words, if $f(T)$ equals $\frac{1}{T}$, the reciprocal of temperature times relative humidity equals absolute humidity.

When the product of $f(T)\times RH$ is absolute humidity in grains per cubic foot, $f(T)$ does not vary as $\frac{1}{T}$ does, but equals the values shown in column 2 of table 5. Consequently, when $\frac{RH}{T}$ (which is the same as $\frac{1}{T}\times RH$) is equated to absolute humidity the resulting values are not in units of grains per cubic foot, or in any other units that can be identified. They are converted into grains per cubic foot, however, if multiplied by the values given in columns 3 and 6 of table 5.

Use of $\frac{RH}{T}$ as a variable was very advantageous in the correlations of this study because (1) when this term is used as a variable the regression equation actually fits the data; (2) the term is an index of absolute humidity; and (3) actual measurements of absolute humidity were not available in a form usable for the analysis, which was made by machine sorts and tabulations of punch cards. Owing to the availability of complete and lengthy records of temperature and relative humidity, great use can be made of the results of a correlation involving $\frac{RH}{T}$ as a variable. One advantage of a method of estimating fire danger through use of this variable is that it makes possible fire-danger ratings, for many stations, for past seasons during which records were made at those stations of air temperature and relative humidity but not of duff moisture.

TABLE 5.—*The values of $f(T)$ in the equation $f(T)\times RH=AH$, and the values of X in the equation $X\left(\frac{RH}{T}\right)=AH$, that give absolute humidities in grains per cubic foot*

Air temperature (T, °F.)	Values that give absolute humidities in grains per cubic foot		Air temperature (T, °F.)	Values that give absolute humidities in grains per cubic foot	
	$f(T)$, in the equation $f(T)\times RH=AH$	X, in the equation $X\left(\frac{RH}{T}\right)=AH$		$f(T)$ in the equation $f(T)\times RH=AH$	X, in the equation $X\left(\frac{RH}{T}\right)=AH$
50	0.04076	2.038	80	0.10934	8.747
55	.04849	2.667	85	.12736	10.826
60	.05745	3.447	90	.14790	13.311
65	.06782	4.408	95	.17124	16.268
70	.07980	5.586	100	.19766	19.766
75	.09356	7.017			

If $\frac{RH}{T}$ is used as an independent variable in the place of relative humidity the gross correlation with duff moisture is equally complete, as is shown by the coefficient r_{XA} in table 6, which gives gross-correlation coefficients for all combinations of duff moisture and the six weather factors having the greatest influence upon it.

TABLE 6.—*Gross-correlation coefficients* [1] *for all combinations of duff moisture (X) on clear-cut area and the six weather factors most important in relation to it*

Correlation coefficient r	Numerical value	Correlation coefficient r	Numerical value	Correlation coefficient r	Numerical value	Correlation coefficient r	Numerical value
XA	+0.672	AB	−0.778	BD	+0.740	DE	−0.007
XB	−.576	AC	−.635	BE	−.020	DF	+.035
XC	−.552	AD	−.657	BF	+.133	EF	−.227
XD	−.535	AE	+.464	CD	+.734		
XE	+.312	AF	−.210	CE	−.053		
XF	−.146	BC	+.784	CF	+.064		

[1] Based on 226 sets of afternoon observations taken 1 or more days after 0.01 inch of precipitation. Explanation of symbols: A=relative humidity divided by air temperature; B=air temperature; C=maximum duff temperature; D=evaporation; E=temperature of the dew point; F=number of days since 0.01 inch of precipitation.

GROSS AND MULTIPLE CORRELATIONS

In an endeavor to find what group of variables bear the closest relation to duff moisture, correlation coefficients were computed for all possible combinations of 2, 3, 4, 5, and 6 weather factors as independent variables with duff-moisture content as the common dependent variable. The multiple-correlation coefficients were computed from the gross r's according to the method outlined by Wallace and Snedecor (*12*). They are given in table 7.

TABLE 7.—*Gross- and multiple-correlation coefficients* [1] *for all combinations of duff moisture (X) on clear-cut area with the six weather factors most important in relation to it*

Correlation coefficient r	Numerical value	Correlation coefficient R	Numerical value	Correlation coefficient R	Numerical value
XA	+0.672	$X.AC$	0.691	$X.ACE$	0.694
XB	−.576	$X.AD$	.683	$X.ACD$	.693
XC	−.552	$X.AB$	.677	$X.ACF$	.692
XD	−.535	$X.AF$	.672	$X.ABC$	.691
XE	+.312	$X.AE$	.672	$X.ADE$	.686
XF	−.146	$X.BE$	.650	$X.ABD$	.684
		$X.CE$	.620	$X.ADF$	.684
		$X.DE$	.617	$X.ABE$	.681
		$X.BC$	.598	$X.ABF$	.677
		$X.BD$	.598	$X.AEF$	.672
		$X.CD$	.584	$X.BDE$	.671
		$X.BF$	.580	$X.BCE$	.666
		$X.CF$	.563	$X.CDE$	.654
		$X.DF$	.550	$X.BEF$	.650
		$X.EF$	.322	$X.CEF$	.623
				$X.DEF$	.620
				$X.BCD$	.608
				$X.BDF$	.604
				$X.BCF$	.604
				$X.CDF$	.596

Correlation coefficient R	Numerical value	Correlation coefficient R	Numerical value	Correlation coefficient R	Numerical value
$X.ACDE$	0.697	$X.ABCDE$	0.697	$X.ABCDEF$	0.697
$X.ABCE$	.694	$X.ACDEF$	.697		
$X.ACEF$	.694	$X.ABCEF$	.694		
$X.ABCD$	.693	$X.ABCDF$	.693		
$X.ACDF$	.693	$X.ABDEF$	.690		
$X.ABCF$	.692	$X.BCDEF$	.677		
$X.ABDE$	.690				
$X.ADEF$	.686				
$X.ABDF$	.684				
$X.ABEF$	.681				
$X.BCDE$	.676				
$X.BDEF$	.671				
$X.BCEF$	.666				
$X.CDEF$	.656				
$X.BCDF$	.615				

[1] Based on 226 sets of afternoon observations taken 1 or more days after 0.01 inch of precipitation. Explanation of symbols: A=relative humidity divided by air temperature; B=air temperature; C=maximum duff temperature; D=evaporation; E=temperature of the dew point; F=number of days since 0.01 inch of precipitation.

TEST OF SIGNIFICANCE OF DIFFERENCE BETWEEN CORRELATION COEFFICIENTS

Table 7 gives evidence that the single variable $\frac{RH}{T}$, or "absolute-humidity index", as it is hereafter called, is associated with duff moisture almost as closely as all six of the independent variables taken together. Evidence to this effect includes the fact that the difference between $r_{X.A}$ and $R_{X.ABCDEF}$ is only 0.025. This difference, when tested according to a method outlined by Russell (*8*), was found to be slightly significant. The procedure follows:

r^2_{XA}=Square of the coefficient of the correlation between duff moisture (X) and relative humidity divided by temperature (A).

$R^2_{X.ABCDEF}$=Square of the coefficient of the multiple correlation between duff moisture (X) and six weather factors.

N=Number of observations.

Term 1=$1-R^2_{X.ABCDEF}$

Term 2=$R^2_{X.ABCDEF}-r^2_{XA}$

If we multiply term 1 by $N\sigma^2_X$ (which is the sum of squares for variable X), the product equals the sum of squares for X independent of the part associated with variables A, B, C, D, E, and F. This sum can be considered as the unexplained sum of squares, or "error." If we multiply term 2 by $N\sigma^2_X$ the product is equal to the sum of squares for X associated with variables B, C, D, E, and F.

The test of significance consists in comparing the variance, or mean square, derived from term 2 with the variance derived from term 1. If the variance of X explained by the variables B, C, D, E, and F is significantly greater than error, then the correlation involving all seven variables is really higher than the simple correlation involving variables X and A.

When such a test of significance is made in practice, the actual values of terms 1 and 2 are used as "relative" sums of squares. Since both terms are multiplied by the same value ($N\sigma^2_X$), their relative values are not changed, and the final test of significance (table 8) is based upon the mean squares.

TABLE 8.—*Computation of relative mean squares to be used in significance test*

Relative sums of squares	Degrees of freedom	Relative mean squares
$1-R^2_{X.ABCDEF}$, or 0.5142	N-7, or 219	0.002348
$R^2_{X.ABCDEF}-r^2_{XA}$, or 0.0342	5	.006840
$1-r^2_{XA}$, or 0.5484	N-2	

The mean squares are obtained by dividing the relative sums of squares by the degrees of freedom associated with them, respectively.

The variance of X associated with B, C, D, E, and F can be compared with error by means of the "z test" developed by Fisher (*1*). The value of z is the difference between the natural logarithms of the two standard deviations; or, in terms of relative mean squares,

$$z=\tfrac{1}{2}\log_e\left(\frac{0.006840}{0.002348}\right)=0.5346$$

The probability, P, of this value's being exceeded by chance is 0.015, as determined from Fisher's tables with the degrees of freedom shown in table 8. (As is shown by Fisher's tables, z=0.3974 and 0.5522 when P=0.05 and 0.01, respectively.)

According to ordinary statistical practice, P must be 0.05 or less if two variances are to be considered significantly different. In more refined tests of significance, P must be 0.01 or less. In this comparison the odds are slightly greater than 1 to 100 that the observed difference is due to chance. While the influence of variables B to F is probably significant, the inclusion of these variables for the sake of a further reduction in the remaining variance of about 3 percent is impractical.

ESTIMATES OF DUFF MOISTURE FROM ABSOLUTE-HUMIDITY INDEX FOR CLEAR-CUT AREA

The regression equation given in the legend of figure 8 and the regression line shown in the figure, which are based on values computed

by standard methods from all available afternoon measurements for the clear-cut area, provide a means of estimating duff moisture content from air temperature and relative humidity combined in the form of the absolute-humidity index. The correlation coefficient for these two variables was found to be $+0.62$; since r^2 equals 0.38, it is evident that only 38 percent of the variation in duff moisture is explained by the absolute-humidity index. Also, the standard error of estimate is ± 2.49 percent. Thus, large errors are necessarily associated with single estimates of duff moisture based on the absolute-humidity index. However, the standard error of an average of estimates varies in inverse proportion with the square root of the number of estimates averaged. Thus an average of 100 estimates of duff moisture has a standard error of ± 0.25 percent, and an average of 50 estimates has a standard error of ± 0.35 percent and hence is acceptable for use in rating the severity of a fire season.

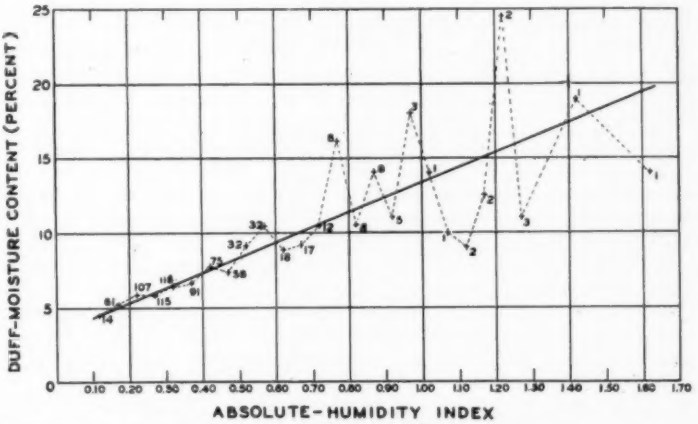

FIGURE 8.—Duff moisture and absolute-humidity index $\left(\frac{RH}{T}\right)$ on clear-cut area. (Basis, all available afternoon data, 793 observations taken at 4:30 p. m.) A=Relative humidity divided by temperature; X=duff moisture content; correlation coefficient ($r_{X.A}$)=+0.62; standard error of estimate ($S_{X.A}$)=±2.49 percent; regression equation is $X=10.00A+3.40$.

The low correlation and its high standard error of estimate preclude any possibility of substituting the simple measurement of temperature and relative humidity for the more difficult measurement of duff-moisture content in field practice. That is, duff moisture cannot be estimated from measurements of these two atmospheric factors with a degree of accuracy satisfactory for forest-protection purposes. Also, this method of estimating is entirely inapplicable on the day of a rain or on the day following a rain.

CORRELATIONS BETWEEN DUFF-MOISTURE CONTENT AND ABSOLUTE-HUMIDITY INDEX FOR HALF-CUT AND FULL-TIMBERED AREAS

Correlations between duff-moisture content and the absolute-humidity index are somewhat lower for the half-cut and full-timbered areas than for the clear-cut area. High fuel moistures tend to prevail on shaded areas. As is seen from figure 5, small changes in temperature and relative humidity have a greater effect on high fuel moistures

than on low ones. As a result, no doubt duff-moisture content is in equilibrium with the weather less often on timbered sites than in the open. Forest cover smooths out rapid fluctuations in weather much less than it retards the drying-out process in the fuels.

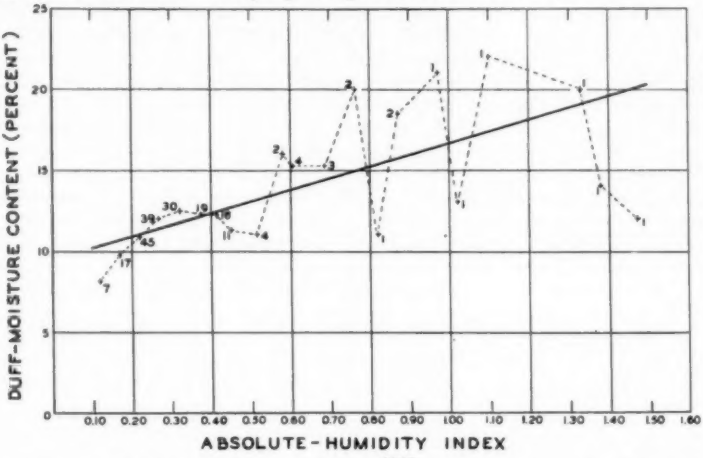

FIGURE 9.—Duff moisture and absolute-humidity index $\left(\frac{RH}{T}\right)$ on half-cut area. (Basis, 208 observations taken at 4:30 p. m. not less than 5 days after 0.01 inch of precipitation.) A=Relative humidity divided by temperature; X=duff moisture content; correlation coefficient ($r_{X.A}$)=+0.47; standard error of estimate ($S_{X.A}$)=±2.78; regression equation is $X=7.25A+9.41$.

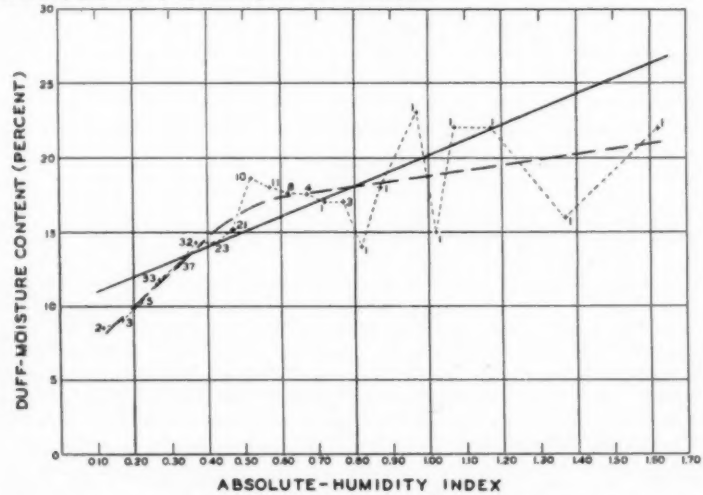

FIGURE 10.—Duff moisture and absolute-humidity index $\left(\frac{RH}{T}\right)$ on full-timbered area. (Basis, 201 observations taken at 4:30 p. m., not less than 6 days after 0.01 inch of precipitation.) For linear regression, correlation coefficient ($r_{X.A}$)=+0.50) standard error of estimate ($S_{X.A}$)=±3.61, and regression equation is $X=10.23A+9.90$; for curvilinear regression, correlation index ($\rho_{X.A}$)=0.59, and standard error of estimate=±3.34 percent.)

Figures 9 and 10 show regressions, correlation coefficients, and standard errors of estimate computed from the afternoon data for

the half-cut and full-timbered sites after elimination of all observations made less than 5 days and less than 6 days, respectively, after 0.01 inch of precipitation.

On inspection of figures 9 and 10 a legitimate question arises, especially in the latter case, as to whether the curve of best fit is a straight line. A curve line was fitted to the data in figure 10 and its correlation index, symbolized by ρ, was found to be $+0.59$. A test of significance performed by Russell's method, previously described, revealed that there is no real difference between the correlation coefficient for the linear regression and the correlation index for the curve. Hence, the fit of the straight-line regression is satisfactory.

Correlations between duff moisture and the absolute-humidity index for the half-cut and full-timbered areas are of little practical value because of the high standard errors of estimate and because such correlations cannot be used until 5 days and 6 days, respectively, after 0.01 inch of precipitation.

CORRELATION OF DUFF MOISTURE WITH ABSOLUTE-HUMIDITY INDEX ON BASIS OF UNRESTRICTED DATA

The most usable method of estimating duff moisture from the absolute-humidity index would be one that could be applied on rainy days and on days immediately following rainy days, as well as at other times. By any method now available, little seems to be gained by making a correlation of duff moisture with the absolute-humidity index on the basis of data including measurements taken during and soon after rain. The effect of such procedure is to make the regression curvilinear. By the addition of free water to duff during rainy periods the duff-moisture content is increased much more than it could be increased by atmospheric humidity, even the increased humidity existing during such periods.

By inclusion of rainy-weather data in the basis of a correlation of duff moisture with absolute-humidity index for the clear-cut area, the correlation coefficient is increased from $+0.62$ to $+0.76$, but the standard error of estimate is increased from ± 2.49 percent to ± 9.19 percent. Thus an average of 100 observations based upon the curve would have the fairly large standard error of ± 0.92 percent.

CORRELATION OF YESTERDAY'S TEMPERATURE AND RELATIVE HUMIDITY WITH TODAY'S DUFF MOISTURE

The fact is well recognized that forest fuels do not respond instantly to sudden changes in the weather. The moisture content of any heavy fuel lags considerably. Since duff is an extremely light and porous material, its moisture content is more closely correlated with atmospheric conditions than that of other forest fuels; but even duff moisture is slower to change than humidity and temperature. A question arises as to whether duff moisture lags enough to justify correlating today's duff-moisture content with yesterday's weather.

The importance of yesterday's temperature and relative humidity as compared with today's in relation to current duff moisture was examined by analyzing the records made on the clear-cut area when the number of days since 0.01 inch of precipitation was 2 or more. In computing the absolute-humidity index different weights were given to the observations taken on the 2 days, respectively. For instance, in one correlation between duff moisture and absolute-humid-

ity index the latter was an average weighted in the ratio of 1 part for yesterday to 3 parts for today. This correlation produced a higher coefficient than any of the other combinations, +0.71; but the improvement in correlation indicated by this coefficient was not large enough to be significant. Hence, it appears that duff moisture on the clear-cut area lags behind the weather but slightly when the number of days since 0.01 inch of precipitation is 2 or more. This restriction on the data has, of course, excluded the influence of most periods of unsettled weather, in other words of most of those days when the greatest lag in duff moisture would be expected.

CORRELATION OF ½-INCH-WOOD MOISTURE AND ABSOLUTE-HUMIDITY INDEX

Two correlations were made between ½-inch-wood moisture and the absolute-humidity index by use of the afternoon measurements taken on the clear-cut area. In the first, all observations made less than 2 days after 0.01 inch of precipitation were rejected. The relation disclosed by this correlation (fig. 11), the regression for which

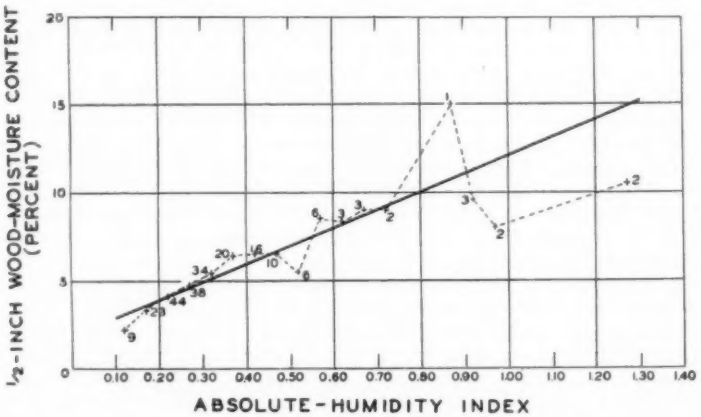

FIGURE 11.—Moisture content of ½-inch wood and absolute-humidity index $\left(\frac{RH}{T}\right)$ on clear-cut area. (Basis, 222 observations taken at 4:30 p. m. 2 or more days after 0.01 inch of precipitation.) A=relative humidity divided by temperature. Y=½-inch-wood moisture content. Correlation coefficient $(r_{Y.A})$=+0.68. Standard error of estimate $(S_{Y.A})$=±1.87. Regression equation is $Y=10.27A+1.83$.

is linear, is slightly closer than that brought out by any of the correlations previously made; the correlation coefficient is +0.68, and the standard error of estimate is ±1.87 percent. In the second correlation, use was made of all available afternoon data for the clear-cut area, including those that had been rejected in the first. As will be seen from figure 12, the regression is curvilinear. This is due almost entirely to the influence of precipitation.

An advantage and a disadvantage are associated with use of either of these two correlations for the purpose of estimating ½-inch-wood moisture from the absolute-humidity index. For estimates based on the curvilinear regression the coefficient was exceptionally high, +0.90, but the standard error of estimate was ±7.46. Thus whereas

an average of 100 estimates based on the linear regression would have a standard error of only ± 0.19, an average of 100 estimates from the curve would have a standard error of ± 0.75. The linear regression applies only when the number of days since 0.01 inch of precipitation is at least 2, but the curvilinear regression can be used for all days. There seems to be little choice between the two, since use of the first is restricted and use of the second permits reasonably accurate estimates only if at least 100 observations are averaged.

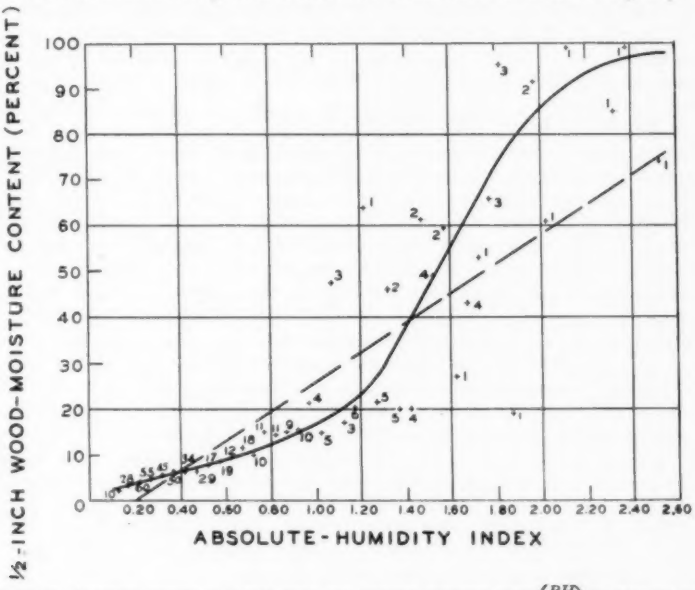

FIGURE 12.—Moisture content of ½-inch wood and absolute-humidity index $\left(\frac{RH}{T}\right)$ on clear-cut area. (Basis, all available afternoon data, 494 observations taken at 4:30 p. m.) For linear regression, correlation coefficient = +0.79, and standard error of estimate = ±10.6 percent. For curvilinear regression, correlation coefficient = +0.90 and standard error of estimate = ±7.46 percent.

RECOMMENDATIONS FOR FURTHER WORK

The results of this analysis suggest several recommendations for further work.

Although in this study correlation of total air movement during the daytime with the moisture content of lightweight fuels revealed no significant relation, it is possible that a significant relation exists between wind velocity during the hours of rapid drying only, say 9 a. m. to noon, and afternoon fuel moisture. The effect of wind should be further investigated.

It might be well, also, to investigate under northern Rocky Mountain conditions some variables not considered in this analysis, such as solar radiation and soil moisture. Results of a study by Stickel (*10*) indicated a slight correlation between these two factors and duff moisture in the Adirondacks. At present satisfactory methods of measuring these variables are wanting, especially in the case of soil moisture.

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Some valuable information might be obtained through study of the correlation between fuel moisture and saturation deficit of the atmosphere; that is, the quantity of water vapor that would have to be added to the atmosphere at a given time in order to produce saturation. This factor provides a measure of the drying power of the atmosphere.

SUMMARY

In efforts toward satisfactory control of the forest fires that do tremendous damage in the United States each year, especially in the northern Rocky Mountain region, forest administrative officers are aided by current knowledge of fire danger—that is, of the sum total of the factors that determine whether fires will start, spread, and do damage. A complete understanding of current fire danger is difficult to obtain, because many factors are involved. By making observations or measurements of several of the most important factors and properly integrating them, however, the forest protectionist can determine the relative fire danger existing at a given time and place. Such a determination guides him in deciding whether and if so how much his force should be temporarily expanded or reduced, and how it should be distributed. In addition, through knowledge of the influence of given factors on fire danger the protectionist is assisted in interpreting weather forecasts and thus in preparing for all classes of fire danger that are likely to arise.

Fuel-moisture content has a very important bearing upon the ease with which fires start and the rate at which they spread. Weather, in turn, is the principal determinant of the moisture content of fuels. Current weather bears an especially close relation to the moisture content of duff and small branch wood, two fuels that are very widely distributed and that carry fire from tree to tree and from log to log. The relation between individual weather elements and the moisture content of these lightweight fuels must be understood before the proper weight can be assigned to each of the many weather factors that contribute to fire danger.

At the Priest River, Idaho, branch of the Northern Rocky Mountain Forest and Range Experiment Station, simultaneous daily measurements have been made, beginning in 1924, of duff-moisture content and the following weather elements and related factors: Maximum, minimum, and current air temperature; temperature of the dew point; current, minimum, and average relative humidity; precipitation, and the number of days since 0.01, 0.10, 0.20, 0.30, and 0.40 inch, respectively, of precipitation; and evaporation. Beginning in 1929, measurements were made also of ½-inch-wood moisture. Wind and maximum duff temperature were added in 1930. These measurements have been made on three adjacent sites in the western white pine type, approximately alike in all respects except that one is clear cut, one-half cut, and one fully timbered.

The fundamental relations between the individual weather elements measured and duff-moisture content for the clear-cut area were examined by means of multiple-correlation analysis. The results of the analysis do not apply to a rainy day or to a day immediately following rain. Data for such days were eliminated to avoid personal errors of fitting freehand curves and to facilitate analysis.

The analysis has definitely shown that of the weather elements and related factors examined current air temperature and relative humidity have the most important effect upon the moisture content of surface duff. Of 14 other weather factors studied, the most important were found to be wind, evaporation, duff temperature, temperature of the dew point, and number of days since 0.01 inch of precipitation. Current air temperature and relative humidity were found to explain all but about 3 percent of the total variance in duff-moisture content associated with those factors and the additional factors just listed. Thus inclusion of the latter in further correlations was impractical.

In this analysis great advantage resulted from the use of the variable $\frac{RH}{T}$, which represents the ratio of relative humidity to air temperature and constitutes an index of absolute humidity. One advantage foreseen in a method of estimating fire danger through use of this variable was that it would make possible fire-danger ratings for the past seasons during which records were made of air temperature and relative humidity but not of duff moisture.

Correlations of duff moisture with relative humidity and air temperature showed errors so large as to preclude all possibility that estimates of duff moisture based on simple measurement of humidity and temperature can be substituted satisfactorily in field practice for actual duff-moisture measurements. Averages for as many as 50 estimates based on these correlations, however, are acceptable for use in rating the severity of a fire season.

Coefficients of correlation of duff moisture with relative humidity and air temperature for the half-cut and full-timbered areas were slightly lower than those for the clear-cut area, and had larger errors associated with them. The usefulness of these correlations is restricted also by the fact that they do not apply until 5 days and 6 days, respectively, after rain.

Coefficients of correlation of ½-inch-wood moisture with relative humidity and air temperature for the three areas differed but little from those of duff moisture.

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THE EFFECT OF DIRECTION OF ILLUMINATION UPON THE VISIBILITY OF A SMOKE COLUMN [1]

By CHARLES C. BUCK, *junior forester*, and WALLACE L. FONS, *junior engineer, California Forest and Range Experiment Station, Forest Service, United States Department of Agriculture* [2]

INTRODUCTION

Preliminary investigations in the detection of forest fires at the California Forest and Range Experiment Station were based on the assumption that the visibility of smoke columns in the field would vary as the visibility of the landscape with varying conditions of atmospheric suspensoid concentration and with the position of the sun. This led to the determination of the effect of direction of the sun with respect to the observer's line of sight upon the visibility of natural features in the landscape through analysis of a large number of lookout observations of natural targets on the Shasta National Forest. Later observations of test-fire smokes, however, indicated clearly that the visibility of smoke columns as reflected in fire-discovery time does not vary in the same manner as landscape visibility. The following laboratory experiment was devised, therefore, to determine in just what manner the visibility of a smoke column varies with different positions of the sun with respect to the observer's line of sight.

MATERIAL AND METHODS

With other factors constant, the visibility of a smoke column varies as a function of its brightness. In order to isolate and measure the variation in visibility due to changes in brightness, the variable influences of background, glare, and atmospheric suspensoid were eliminated from the experimental set-up.[3] The two elements primarily concerned in the relationships to be determined, then, were a column of smoke and a light beam of parallel rays.

Figure 1 shows the arrangement in plan and elevation of the equipment as it was set up for the experiment. The beam of parallel rays was produced by a 400-watt projection lantern (*a*) with the projection lens removed, operating on an alternating-current circuit. A wooden frame in the form of a tank, 3 feet deep and 12 feet in diameter, was built and set 1 foot above the floor. This tank was

[1] Received for publication June 15, 1935; issued February 1936.

[2] The authors wish to acknowledge indebtedness to A. A. Brown, associate silviculturist at the California Forest and Range Experiment Station, who initiated the experiment, made arrangements for obtaining the laboratory in which it was performed, and gave considerable time to the general supervision of the work. They also wish to thank Professor Minor of the optometry division of the University of California for his interest and for suggestions offered in organizing the experiment, and Professor Boelter of the engineering department of the university for his cooperation and for his kindness in offering criticisms of the original manuscript.

[3] Certain conclusions, however, relative to the effect of atmospheric suspensoid material upon visibility have been drawn from the laboratory observations.

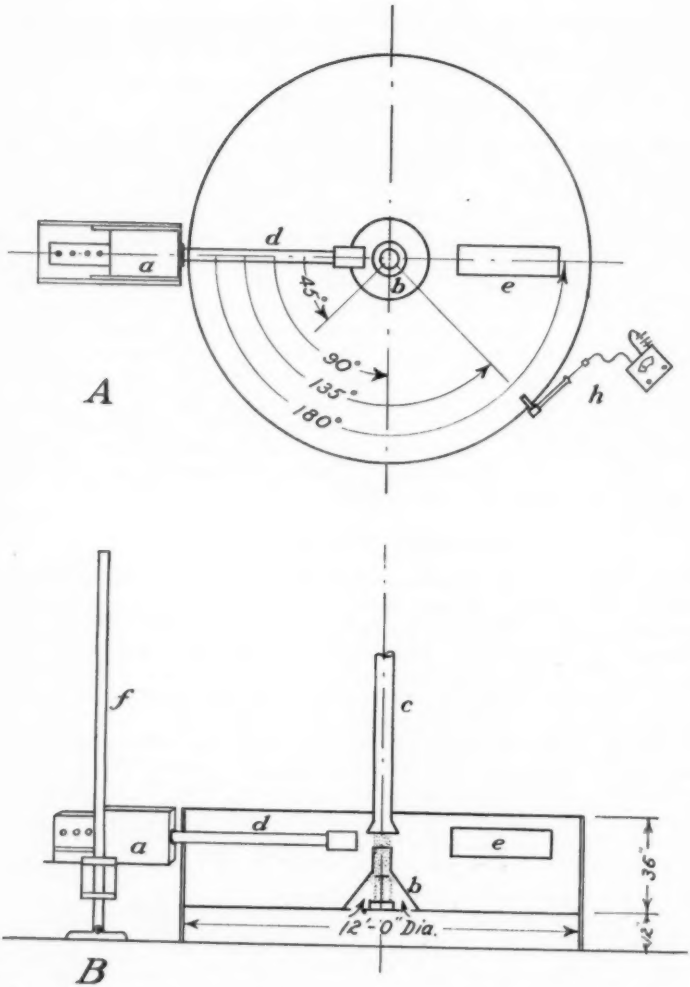

FIGURE 1.—Arrangement of experimental apparatus: *A*, Plan; *B*, elevation. Explanation in text.

lined with black builder's felt to eliminate variations in background and minimize the reflection of stray light. In the center of the tank was placed an inverted funnel-shaped hood (*b*) in which the smoke was produced. Eight inches above the top of the hood was placed a flue (*c*) to carry off the smoke and create a draft to keep the smoke in a uniform column. A specially constructed tube (*d*) was placed in front of the projector to adjust the size of the light beam and absorb the small amount of nonparallel rays produced by the projector. The beam of light was directed through the side of the tank at a height 2 inches above the top of the hood in the center of the tank. An 8-inch tube 3 feet long with one end closed (*e*) was hung in line with the beam of light to intercept it after it had passed through the smoke column, thus eliminating any reflection from the opposite side of the tank. The projector was mounted on an elevating frame (*f*) so that the light source could be adjusted to correspond with various elevations of the sun. All equipment was painted with flat black paint and observations were made in a totally darkened room.

The smoke column was produced by burning Chinese punk, the desired concentration of smoke being obtained by varying the number of punks burned at one time. A baffle plate at the lower end of the neck of the hood broke up the smoke and produced a uniform column 2¼ inches in diameter regardless of the number of punks burned. The punk burned at a uniform rate and produced a smoke similar in color to actual smokes observed in the field.

The diameter of the light beam was adjusted to the diameter of the smoke column so the apparent illuminated area of the smoke remained approximately the same regardless of the position from which it was observed.

A Macbeth illuminometer was used to measure the brightness of the illuminated smoke column. Measurements of smoke visibility given are based on the brightness of the smoke measured with this instrument in international candles per square foot.

Measurements of brightness and visibility of the illuminated smoke column were made at observation stations placed at intervals of 22½° around the tank in the same horizontal plane as the illuminated portion of the smoke column. These stations are designated by the angle *abh* in the diagram, in which the illuminometer (*h*) is shown at station 135°.

EXPERIMENTAL DATA

In the preliminary laboratory tests, observations with the Bennett-Casella visibility meter,[4] used for visibility measurements in the field, were made simultaneously with observations with the Macbeth illuminometer in order to establish the relationship between the brightness of the smoke and the visibility-meter readings with a black background. Table 1 is a summary of the average brightness

[4] BENNETT, M. G. A VISIBILITY METER. Jour. Sci. Instruments 8 (4): 123–126. 1931.

values obtained. In figure 2 visibility-meter readings are plotted over these values on a logarithmic scale. Since the plotted points fall along a straight line it follows that the visibility-meter readings are a simple multiple of the logarithm of the brightness.

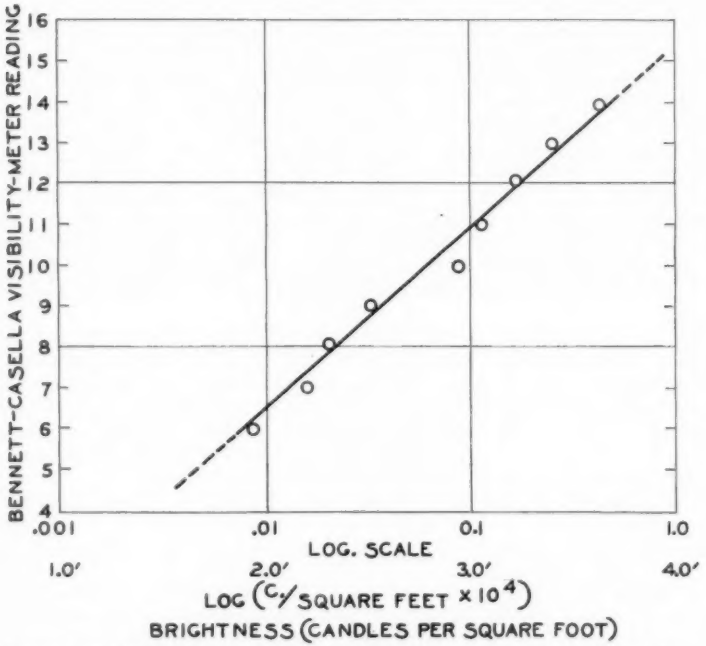

FIGURE 2.—Relationship of Bennett-Casella visibility-meter reading to brightness of smoke column.

TABLE 1.—*Relationship between Bennett-Casella visibility-meter readings and brightness measurements of smoke columns*

Visibility-meter reading	Brightness (candles per square foot)	Log $\left(\frac{\text{candles}}{\text{square foot} \times 10^4}\right)$
6	0.0087	1.940
7	.0158	2.199
8	.0198	2.297
9	.0314	2.497
10	.0825	2.916
11	.1140	3.057
12	.1680	3.225
13	.2490	3.396
14	.4210	3.624

There is a decided inequality in the graduations of opacity of the various filters in the visibility meter, resulting in the failure of the plotted values in figure 2 to fall exactly on a straight line. This inequality in the steps of opacity also gives visibility-meter readings

slightly too low for low values and approximately the same amount too high for high values (fig. 3). The approximate relationship indicated, however, when brightness is expressed in candles per square foot, is—

$$V=3.6\left[\log \frac{(\text{candles})}{(\text{square foot})}\times 10^4\right]$$

where V is visibility-meter reading.

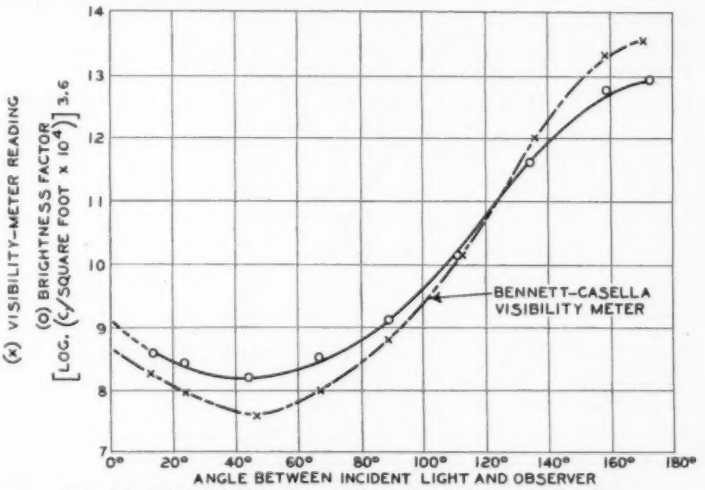
(x) VISIBILITY-METER READING
(o) BRIGHTNESS FACTOR
[LOG. (c/SQUARE FOOT x 10⁴)] 3.6
BENNETT-CASELLA VISIBILITY METER
ANGLE BETWEEN INCIDENT LIGHT AND OBSERVER

FIGURE 3.—Effect of direction of illumination on the visibility of a smoke column.

In the preliminary observations a large number of brightness measurements was made with the line of sight in a horizontal plane and the light source in positions varying from horizontal to a vertical

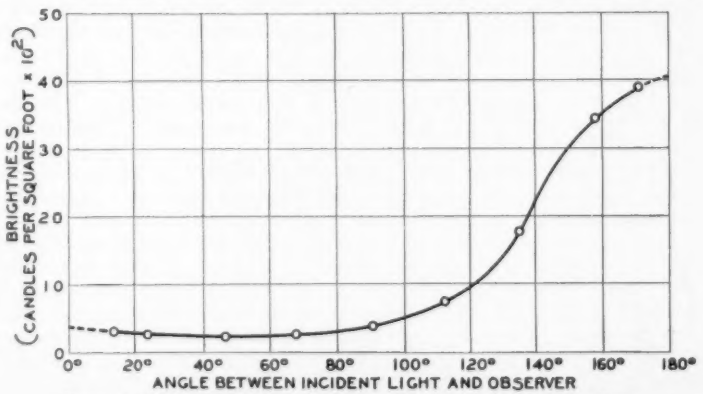

FIGURE 4.—Effect of direction of illumination on the brightness of a smoke column.

angle of 50° with respect to the smoke column, and the effect of direction of illumination on the brightness of the smoke column was

determined. Considerable variation was noted in brightness measurements taken at different times, resulting in differences in the numerical values obtained. A representative set of measurements is given in table 2 and shown graphically in figures 3 and 4. The angles indicated are the angles measured at the smoke between the light source and the observer. Visibility-meter readings given are determined from the brightness measurements through the relationship shown in figure 2. This was done to smooth out the roughnesses in individual visibility-meter readings.

TABLE 2.—*Effect of direction of illumination upon the brightness of a smoke column* [1]

Station (degrees)	Illuminometer reading [2]	Brightness (candles/square foot)	Log ($B \times 10^4$)	[Log ($B \times 10^4$)] $\times 3.6$	Visibility-meter reading
0.0		0.0300	2.477	8.9	8.6
11.25	3.19–49	.0249	2.396	8.6	8.2
22.5	2.76–49	.0216	2.334	8.4	8.0
45.0	2.24–49	.0176	2.246	8.1	7.6
67.5	2.81–49	.0220	2.342	8.4	8.0
90.0	4.23–49	.0331	2.520	9.1	8.8
112.5	8.71–49	.0684	2.835	10.2	10.2
135.0	6.16–12	.1710	3.233	11.6	12.0
157.5	12.30–12	.3410	3.533	12.7	13.3
170.0	14.05–12	.3890	3.590	12.9	13.5
180.0		.4000	3.602	13.0	13.6

[1] Smoke density=5 punks; vertical angle: incident light=0°, observation=0°. Variation in illumination at smoke column=91.6 to 90.0 foot-candles. Tabulated values in column 2 are averages of values taken at plus and minus horizontal angles; values for 0° and 180° in column 3 are extrapolated; values in column 6 taken from figure 2.

[2] Filter no. 49—candles/square foot=reading $\times$ 0.00784; filter no. 12—candles/square foot=reading $\times$ 0.0277.

Several sets of observations resulted in variations in the numerical values of brightness obtained, but the trends of increasing brightness with increasing angle were all of the same shape. These variations were traced to two varying factors in the set-up: Light intensity of the source, affected by changing voltage in the light circuit; and density of the smoke column, affected by changes in the draft up the flue, which depended both upon outside air temperature and temperature of the room. To eliminate these variations, a voltmeter was placed in the light circuit and a photronic cell[5] in the light beam behind the smoke column. It was assumed that when the voltage remained constant any variation in the reading of the photronic cell was caused by variation in the smoke density. In the final observations readings were taken only when the voltmeter and photronic cell measured constant values.

After the trend of brightness had been established for various angles between the light source and the observer with both in a horizontal plane, several series of observations were made with the line of sight of the observer in a horizontal plane but with the light source raised to various vertical angles to correspond with the varying elevations of the sun. The results obtained from these observations did not check with computed values, for the reason that elevating the light source increased the volume of smoke illuminated, thus increasing its apparent brightness. In the field, where the entire smoke column is in sunlight, an increase in the elevation of the sun does not increase the volume of smoke illuminated. With the light source in a horizontal plane, a series of measurements of brightness was made from

[5] GOODWIN, W. N., JR. THE PHOTRONIC ILLUMINATION METER. Illuminating Engin. Soc. Trans. 27: 823-835, illus. 1932.

various vertical angles at different horizontal stations. The values thus obtained corresponded with the values obtained in a horizontal plane at an angle equal to the "spherical" angle between the light source and the observer. These observations indicated that the angle measured at the smoke column between the light source and the observer determines the effect of direction of illumination on the visibility of a smoke column regardless of the azimuths and elevations of any of the three points. All later observations were taken in a horizontal plane, since the horizontal angles between light source and observer could be substituted for "spherical" angles directly. In figure 5 is shown the effect of direction of illumination for different

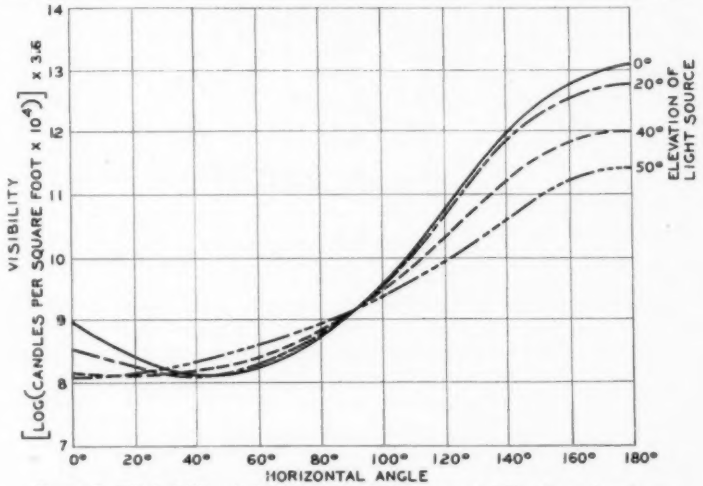

FIGURE 5.—Effect of different elevations of the light source on visibility of a smoke column.

elevations of the light source. Table 3 gives the equivalent angles for different horizontal and vertical components.

TABLE 3.—*Angle between light source and observer for given horizontal and vertical angle components*

Horizontal angle (degrees)	Equivalent for vertical angle (degrees) indicated									
	5°	10°	15°	20°	25°	30°	35°	40°	45°	50°
0	5	10	15	20	25	30	35	40	45	50
10	12	15	19	23	27	32	37	42	47	52
20	21	23	26	29	32	36	40	45	49	54
30	31	32	34	36	39	42	46	50	54	57
40	41	42	43	45	47	50	52	55	57	61
50	51	52	53	54	55	57	59	61	63	66
60	60	61	61	62	63	65	66	68	70	72
70	70	71	71	72	72	73	74	75	76	78
80	80	80	80	81	81	82	82	83	83	84
90	90	90	90	90	90	90	90	90	90	90
100	100	100	100	99	99	99	99	98	98	97
110	110	110	110	109	109	108	108	106	105	104
120	120	120	120	119	118	116	115	113	112	110
130	130	130	129	128	126	124	122	120	118	116
140	140	140	139	137	135	132	130	127	124	122
150	150	150	148	145	143	140	137	134	130	125
160	160	160	157	153	150	146	142	138	134	128
170	170	170	167	162	158	150	146	140	136	130
180	175	170	165	160	155	150	145	140	135	130

Measurements of the effect of concentration of the smoke column on its brightness and on its light-transmission factor were made at

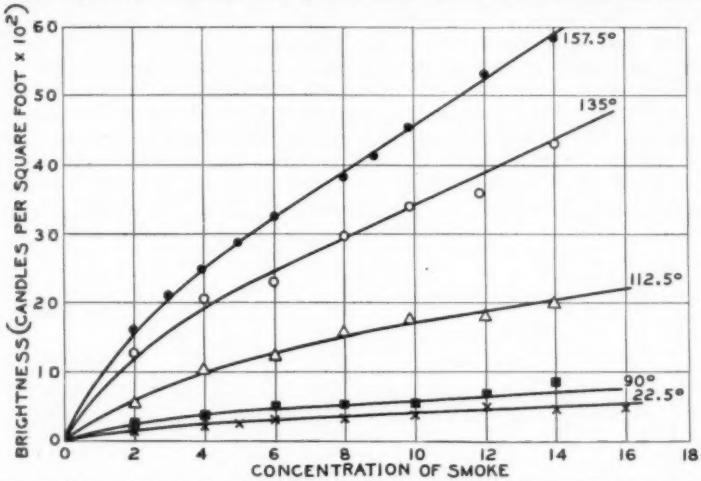

FIGURE 6.—Effect of concentration of smoke on its brightness for different angles (indicated on curves) between (horizontal) light source and observer.

the same time. The concentration of smoke was varied by placing additional punks under the hood, the number ranging from 1 to 14. The heat caused by more than this number produced an exceptionally

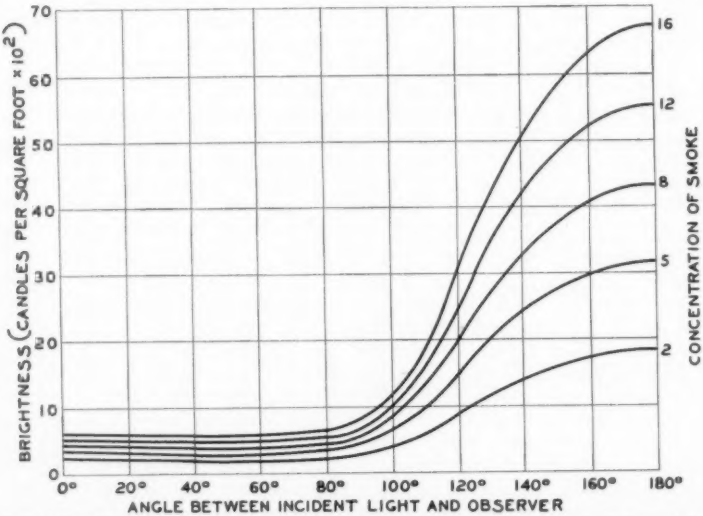

FIGURE 7.—Effect of direction of illumination on brightness of a smoke column for different concentrations of the smoke.

strong draft, which resulted in discrepancies in both brightness and light-transmission measurements. Readings of brightness were taken

at five stations for each change in concentration of the smoke (table 4). These values are shown graphically in figure 6. Figure 7 is derived from figure 6 by cross-plotting the five curves in the latter.

The photronic cell was used to record the foot-candles of light passing through the smoke column. For each change in the concentration of the smoke, as determined by the number of punks burned, the meter of the photronic cell was watched, and the reading that seemed to represent the most stable and uniform condition of the smoke was tabulated. These values for the various concentrations are given in table 5 with their corresponding percentages of light transmission, which are presented graphically in figure 8.

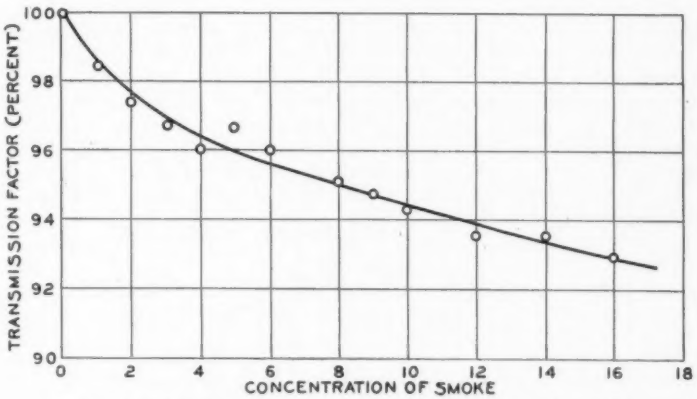

FIGURE 8.—Effect of concentration of a smoke column on its transmission of light.

TABLE 4.—*Effect of concentration of the smoke on the brightness of a smoke column at different angles between light source and observer* [1]

Concentration of smoke column (number of punks)	22.5°		90°		112.5°		135°		157.5°	
	Illuminometer reading	Brightness [2]	Illuminometer reading	Brightness [2]	Illuminometer reading	Brightness [2]	Illuminometer reading	Brightness [2]	Illuminometer reading	Brightness [2]
2	1.9 –49	0.0149	3.3 –49	0.0259	2.1–12	0.0582	4.6 –12	0.128	6.0 –12	0.166
3	3.3 –	.0259	4.0 –	.0314					7.75–	.215
4	3.0 –	.0236	4.7 –	.0368	4.0–	.1110	7.75–	.215	9.0 –	.250
5	3.55–	.0279	5.3 –	.0416					10.6 –	.293
6	3.6 –	.0282	5.37–	.0421	4.6–	.1280	8.3 –	.230	11.75–	.325
8	4.4 –	.0345	5.8 –	.0455	6.1–	.1690	10.6 –	.294	13.4 –	.372
9	5.6 –	.0439	6.75–	.0530					15.0 –	.416
10	4.4 –	.0345	6.8 –	.0532	6.1–	.1690	12.4 –	.344	16.5 –	.457
12	6.9 –	.0541	9.0 –	.0706	6.5–	.1800	12.6 –	.349	19.2 –	.532
14	6.2 –	.0486	11.5 –	.0902	7.1–	.1970	15.4 –	.427	6.5 $-\frac{12}{49}$	.586
16					7.3–	.2020				
18							18.0 –	.498		

[1] Illumination at smoke column: 130.0 foot-candles.

[2] $\frac{\text{Candles}}{\text{Square foot}}$.

TABLE 5.—*Effect of concentration of a smoke column on transmission of light*

Concentration of smoke column (number of punks)	Light transmitted	Transmission factor	Concentration of smoke column (number of punks)	Light transmitted	Transmission factor	Concentration of smoke column (number of punks)	Light transmitted	Transmission factor
	Foot-candles	*Percent*		*Foot-candles*	*Percent*		*Foot-candles*	*Percent*
0	33.1	100.0	5	32.0	96.7	10	31.2	94.3
1	32.6	98.5	6	31.8	96.0	11		
2	32.2	97.4	7			12	31.0	93.6
3	32.0	96.7	8	31.5	95.1	13		
4	31.8	96.0	9	31.4	94.8	14	31.0	93.6

No measurements were made of the effect of suspensoid in the atmosphere, since there was no way in which the suspensoid could be illuminated uniformly in the laboratory. The foot-candles of light from a point light source decrease as the square of the distance from the source; hence the atmospheric suspensoid would be much more strongly illuminated near the light source than at some distance from it. This relationship was checked in the laboratory. For the same reason no observations were made with illuminated backgrounds.

It was observed that the veiling brightness of the illuminated atmospheric suspensoid increased in the same manner as the brightness of the smoke column when the angle of observation with respect to the light source was increased. Incidentally it was noted that a smoke column illuminated only by diffused light was visible through a considerable density of atmospheric suspensoid as long as the line of sight through the suspensoid was in shadow. When the line of sight passed through a short distance of illuminated suspensoid, however, the smoke column became decidedly less visible even though the observer was in shadow. The observer here remained in shadow to eliminate the effect of glare.

DISCUSSION

Before any general conclusions can be drawn, some explanation of the various measurements of brightness and visibility must be made. In the laboratory, color contrasts were not considered and background was held constant with a brightness of zero. The visibility of the illuminated smoke column was therefore a function of its brightness.

According to Fechner's law, the eye reacts to variations in the stimulus as the logarithm of the stimulus. Thus the visibility of the smoke column varies as the logarithm of the brightness. A comparison of figures 3 and 4 indicates the relationship between the brightness of the illuminated smoke column and its visibility. The brightness measured in absolute units in figure 4 increases, from a minimum at an angle between light source and observer of 45°, to approximately 19 times this value at an angle of 170°. In figure 3, however, when the logarithmic values are plotted, it is seen that the visibility increases only by approximately 62½ percent in the same interval. A further study of figure 4 indicates that Bennett-Casella visibility-meter readings are approximately in proportion to the visibility of the smoke column.

Some consideration should be given to the cause of the phenomenon of increasing brightness with increasing angle between the light source and line of sight of the observer, as illustrated in the curves of figure 7. The brightness of the smoke column observed at angles of less than 90° is caused principally by light reflected by the smoke particles, while at angles greater than 90° the brightness is caused largely by transmitted scattered light. Since the smoke column transmits much more light than it reflects, the brightness of the column is considerably greater when observed from the larger angles. Light incident on a smoke column is either reflected or scattered and at least partially polarized, depending on the size and character of the individual smoke particles; and the light reflected or scattered by each particle may be reflected or further scattered many times by additional particles in its path. The minimum brightness measurements obtained were recorded at station 45°, the sum of the multiple reflected and scattered light being at a minimum at this point. This causes the curves to take the form of a trigonometric function of the angle between light source and observer 45° out of phase.

The phenomenon of increasing brightness of smoke with increasing angle between the sun and observer is of importance because it not only determines the relative intrinsic visibility of a column of smoke as determined by the direction from which the smoke is observed, but has a strong influence on the detrimental effect on visibility of haze in the form of solid suspensoid material in the atmosphere.

In addition to producing a veiling brightness which lowers visibility by decreasing the contrast between the smoke column and its background, suspensoid material in the atmosphere also decreases the visibility of an object by decreasing the transmission of light reflected from the object through the atmosphere to the observer. Figure 8 illustrates the manner in which this transmitted light is decreased by various concentrations of suspensoid material in a smoke column. It is assumed here that light transmission through a longer distance of less concentrated suspensoid would follow the same trend as that indicated for the smoke column. Further laboratory work is now being planned to determine the relative effects of concentration and distance upon the transmission of light through an atmosphere containing suspensoid material. The relatively high light-transmission factor of a smoke column obtained in the experiment indicates that in general the detrimental effect on visibility of an atmospheric suspensoid is probably more attributable to veiling brightness than to decreased light transmission.

CONCLUSIONS

Although it is unlikely that relationships observed in the laboratory would ever be as sharply defined in the field, where a large number of variable factors is inevitably encountered, the same trends will be followed. The results of a series of laboratory experiments to determine the effect of certain factors on the visibility of a smoke column may be given, then, in terms of field observations, as follows:

(1) The angle measured at the smoke between the sun and the observer determines the effect of direction of illumination on the visibility of a smoke column regardless of its azimuth or position of the sun as determined by the time of day.

(2) In an atmosphere containing little or no suspensoid material, the visibility of a smoke column is influenced appreciably by the relative position of the sun with respect to the line of sight of an observer. There is little variation in the visibility of the smoke column with changes in the angle between the incident light and line of observation from 0° to 90°—that is, with the sun varying in position from directly behind the observer to a line at right angles with his line of sight. When the angle is greater than 90° there is a very marked progressive increase in the visibility of the smoke until the observer is looking almost directly into the sun. The smoke is not visible when the sun forms its background.

(3) When there is an appreciable amount of suspensoid material in the atmosphere there is a decrease in the visibility of a smoke column viewed through the atmosphere owing to a combination of two factors—decreased light transmission factor of the atmosphere and veiling brightness of the suspensoid material. The first factor is constant for a given suspensoid concentration, while the second is present only when the suspensoid is illuminated and varies not only with suspensoid concentration but also with direction of illumination with respect to the observer's line of sight.

(4) The visibility of an object is decreased when the concentration of atmospheric suspensoid through which the object is viewed is increased. When the sun is behind or at right angles to the observer's line of sight, this decrease in visibility may be caused largely by decreased light transmission of the atmosphere, but with angles between the sun and observer greater than 90°, the detrimental effect of veiling brightness on visibility is much greater.

(5) The increasing brightness of suspensoid particles with increasing angle between the sun and observer's line of sight definitely affects the ease with which fires may be detected by lookout observers. When there is present an appreciable concentration of atmospheric suspensoid material, the visibility of landscape features in the general direction of the sun is decreased by the veiling brightness of the suspensoid material. The same factor, however, which causes the increased brightness of the atmospheric suspensoid also increases the brightness of a smoke column observed in that direction. The net result, then, is that even with a relatively high concentration of atmospheric suspensoid material present, smoke columns may be detected more readily and at greater distances in the general direction of the sun than in the opposite direction.

TOXICITY OF ALUMINUM SALTS TO TOBACCO PLANTS[1]

By WALTER S. EISENMENGER

Head of Department of Agronomy, Massachusetts Agricultural Experiment Station

INTRODUCTION

It has been suggested that certain diseases which affect tobacco when it is grown after corn, timothy, or red clover may have their origin in the soluble aluminum salts of the soil. The principal difficulty in such rotations has been the development of the so-called "brown root rot" of tobacco. The present study was undertaken to determine whether soluble aluminum salts have the capacity to engender this disease.

The relatively large amount of aluminum in the earth's crust suggests that a considerable amount of this element would be found in the soil solution. However, the extreme insolubility of aluminum silicates prevents a rapid accumulation of aluminum in the ionic state. This means that aluminum salts of the weak acids are not highly soluble. Thus it may be anticipated that conditions for soluble aluminum would be more favorable when the hydrogen-ion concentration of the soil is high and the concentration of other salts usually found in soil is low.

EFFECT OF ALUMINUM ON TOBACCO PLANTS GROWN IN SOLUTIONS

Tobacco planted on September 18, 1934, and harvested on December 6, 1934, was grown in a complete nutrient solution (Knop's solution) on alternate 2 days, and during the intervening 2 days in various solutions of aluminum citrate. The containers were of 1-quart capacity. During the entire period Knop's solution with a trace of boric acid was used as the standard and in this the controls were grown. Five plants were grown in each solution. The quantity of aluminum taken up by the roots of the plants in the different solutions was as follows:

Percentage proportions of 0.006 molar aluminum citrate, added to culture solutions	Parts per million of aluminum in roots of tobacco plants	Percentage proportions of 0.006 molar aluminum citrate, added to culture solutions	Parts per million of aluminum in roots of tobacco plants
Control	3	50	503
0[1]	1	70	569
2	6	85	597
5	12	95	893
15	361	98	2,165
30	502	100	2,165

[1] Zero indicates distilled water.

Plants grown in aluminum citrate showed definite signs of toxicity. The general toxic effect was more pronounced in the roots; but, as

[1] Received for publication Apr. 23, 1935; issued February 1936. Published as contribution no. 211 of the Massachusetts Agricultural Experiment Station.

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compared to the controls, the tops showed a slightly more retarded growth than the roots. The most marked retarding influence of aluminum citrate began to be apparent in concentrations between 0.0009 M and 0.0018 M. Among the plants in other experiments, including plants in the seedling stage, the decrease in growth rate was more rapid at the above-mentioned concentrations. Thus the retardation in growth rate was greater with the percentages between 15 and 30 percent of the total concentration 0.006 M; that is, approximately 24 to 48 parts per million of aluminum. At concentrations higher than this the plants showed only moderately increased effects of toxicity up to 162 parts per million, or 100 percent of 0.006 M. McLean and Gilbert[2] found that aluminum in aluminum citrate, in quantities from 3 to 13 parts per million, was stimulating to plant life, while in larger amounts it was toxic.

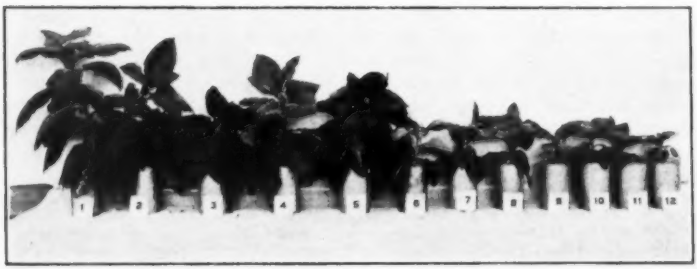

FIGURE 1.—Tobacco plants grown for alternating 2-day periods in complete nutrient solutions and in water containing various percentage proportions of 0.006-molar aluminum citrate solution: No. 1, control; no. 2, 0 (distilled water); no. 3, 2; no. 4, 5; no. 5, 15; no. 6, 30; no. 7, 50; no. 8, 70; no. 9, 85; no. 10, 95; no. 11, 98; no. 12, 100 percent of 0.006-molar aluminum citrate solution.

The plants grown in distilled water in alternate 2-day periods and in a complete nutrient solution in the intervening 2-day periods, showed retarded growth rate as compared with the plants grown in a complete nutrient solution during all the days of experimentation. The plants which received aluminum in relatively small amounts, as 0.00012 M, showed no stimulation of growth because of the presence of aluminum.

The plants affected by aluminum acquired a darker foliage than normal plants and the tops were not as large (fig. 1). These tops did not appear to be diseased or abnormal like those of plants influenced by single ions of the alkalies, or alkali earths, but appeared to be suffering from a lack of nutrients. The toxicity of the aluminum to the root was manifested by the retardation of growth of the primary root, and in cases of more definite and severe toxicity the lesion became so intensified that it assumed the nature of an abscission with tissue sloughing off and lateral roots taking the place of the primary one. In cases of more marked injury of root tip, when the solution was changed from aluminum only to complete nutrient solution, a white flocculent substance formed on the end of the injured root. This was apparently in part a phosphate or hydrosol of aluminum formed from the drop adhering to the injured root from the previous aluminum

[2] MCLEAN, F. T., and GILBERT, B. E. ALUMINUM TOXICITY. Plant Physiol. 3: 293-302. 1928.

solution and in part an exudate from the injured tissue. The aluminum is apparently located in the superficial layers of the root and eventually leads to reduced intake of nutrients.

Although local brown areas were observed, there was no general browning of the roots as is the case when brown root rot occurs in fields. In other words, there appeared to be no relation between the toxic effect of aluminum and the symptoms of brown root rot.

The lethal dose of aluminum citrate for tobacco is not known. The plants when removed from the toxic solution and placed in a more favorable medium seem to have marked capacity for recovering. Lateral roots are thrown out above the water line of the nutrient solution, and only the portion in the toxic solution seems to be affected.

It would seem that the presence of excessively large amounts of aluminum in plants grown in solutions of relatively high concentrations of aluminum citrate is due in part to imbibition only to areas characterized as superficial layers of tissue.

It is apparent from the work of previous investigators, and from the present investigation, that aluminum occurs in greater abundance in the roots than in the tops. With this in mind an analysis was made of aluminum in the roots of tobacco plants grown at the different aluminum concentrations shown on page 519. The method used was that of Winter, Thrun, and Bird,[3] according to which the colors developed in the presence of aurintricarboxylic acid are compared. It was found that much aluminum was taken up by the plants growing at high concentrations of aluminum (fig. 1). The high values of aluminum in the plants began when they were grown at 15 percent of the 0.006 molar concentration, and this is the percentage at which the growth curve begins to fall perceptibly. At lower concentrations there was no great toxicity. At 15-percent concentration there was present in the solution an approximate total of 24 parts per million of aluminum. At the extreme high concentration there was a large intake of aluminum without a subsequent pronounced lowering of the growth curve. The tops of the plants contained less aluminum than the roots, but the amounts did not seem to be in proportion to the quantity added to the solution. The small amount of aluminum found in the plants grown in distilled water and in the controls may probably be accounted for by the fact that these plants had grown in soil before they were transplanted, and had taken up aluminum from the soil at a very early stage of growth.

EFFECT OF CALCIUM IN CORRECTING THE TOXICITY OF ALUMINUM TO TOBACCO PLANTS GROWN IN SOLUTIONS

It has been assumed that ionic calcium has the capacity to counteract the unfavorable effects induced by an unbalanced proportion of other ions. To determine the validity of this assumption a series of tests was made. Calcium nitrate and aluminum citrate were used in varying proportions in a complete nutrient solution. Aluminum citrate does not readily precipitate over a wide range of hydrogen-ion concentrations. There were eight sets of molecular proportions of the two salts calcium nitrate and aluminum citrate as shown in table 1, which gives the percentage proportions of 0.006 M of each salt.

[3] WINTER, O. B., THRUN, W. E., and BIRD, O. D. THE DETERMINATION OF ALUMINUM IN PLANTS. I. A STUDY OF THE USE OF AURINTRICARBOXYLIC ACID FOR THE COLORIMETRIC DETERMINATION OF ALUMINUM. Jour. Amer. Chem. Soc. 51: 2721–2731, illus. 1929.

The test plants were grown in solutions which were like those of the controls except that they contained varying amounts of aluminum and calcium, and four times as much potassium nitrate. Thus, 0 to 100 percent of aluminum citrate and an inverse percentage proportion of calcium nitrate. The plants used as controls were grown in Knop's solution without modification. The solutions were changed twice a week. The age of plants when harvested was 2½ months. Five plants were used for each concentration.

In those solutions containing the maximum percentage proportions of aluminum citrate the reaction was approximately equivalent to a pH of 4, while those with minimum amounts of the salt had a pH equivalent to about 5.9.

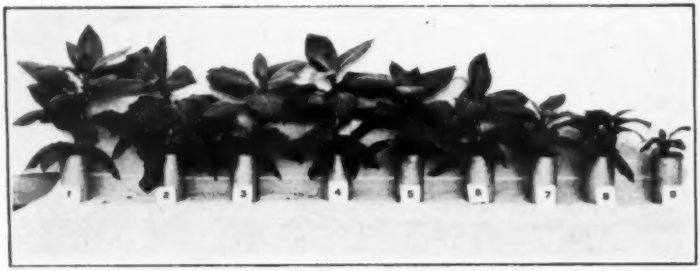

FIGURE 2.—Tobacco plants grown in medium in which only calcium nitrate and aluminum citrate were varied, the other nutrients being kept constant. The following numbers express the relative percentage proportions of 0.006 molar calcium nitrate and of 0.006 molar aluminum citrate used: 1, Control; 2, 100 percent calcium nitrate+0 percent aluminum citrate; 3, 95 percent calcium nitrate+5 percent aluminum citrate; 4, 85 percent calcium nitrate+15 percent aluminum citrate; 5, 50 percent calcium nitrate+50 percent aluminum citrate; 6, 25 percent calcium nitrate+75 percent aluminum citrate; 7, 15 percent calcium nitrate+85 percent aluminum citrate; 8, 5 percent calcium nitrate+95 percent aluminum citrate; 9, 0 percent calcium nitrate+100 percent aluminum citrate.

The plants receiving no calcium were stunted, as indicated by their roots and tops. Where a small amount of calcium was added (0.0003 M $Ca(NO_3)_2$), the tops were approximately 3 times as tall as those of plants in solutions containing no calcium, and the dry weight of these plants was 9 times that of those in the calcium-free solution.

The most perceptible effect of low calcium on the tops was the retarded development of the primordial meristem. The leaves at the apex of the plant seemed to unfold with difficulty. The color was darker than that of normally growing plants, indicating that the roots were not permitting the intake of the small amount of calcium that was present. The roots of the plants that grew in the highest amount of aluminum had abscissions at their ends, and to these ends a flocculent precipitate adhered. It would seem that the aluminum was taken in and precipitated and then the degeneration of tissue took place (table 1 and fig. 2).

It is apparent that plants grown in soil or water solutions do take up an appreciable amount of aluminum. Where aluminum is present even in moderate quantities, the plants, especially the roots, contain far more proportionately than is present in a soluble state in the soil.

TABLE 1.—*Growth of tobacco plants as affected by various molar proportions of aluminum citrate and calcium nitrate*

Percentage proportions of 0.006 molar aluminum citrate and calcium nitrate used		Average height and weight of tobacco plants expressed as percentages of the growth in standard solution		Percentage proportions of 0.006 molar aluminum citrate and calcium nitrate used		Average height and weight of tobacco plants expressed as percentages of the growth in standard solution	
Aluminum citrate	Calcium nitrate	Height	Weight	Aluminum citrate	Calcium nitrate	Height	Weight
100	0	23	6	50	50	86	74
95	5	58	34	15	85	95	101
85	15	75	59	5	95	100	105
75	25	76	63	0	100	90	90

Unless other factors obtain, such as the presence of an excessive amount of organic matter or phosphates, a large amount of aluminum occurs with a high concentration of hydrogen ions. It is not clear whether this is the result of hydroloysis of aluminum compounds, or whether a reaction is initiated by the action of hydrogen ions upon the otherwise rather insoluble aluminum compounds. Energy factors bringing about equilibrium of reaction need to be considered.

AMOUNT OF SOLUBLE ALUMINUM IN SOILS IN WHICH TOBACCO, CORN, TIMOTHY, AND CLOVER HAD BEEN GROWN

The aspect of brown root rot of tobacco in soils which are subject to rotation is well recognized in the Connecticut Valley. It is not uncommon to find the disease when tobacco is planted after timothy, clover, or corn, but not on land where tobacco has grown for several years in succession.

Although there is not, to the writer, a rational basis for supposing that different crops increase the amount of available aluminum in the soil, analyses were made of soils of the same type which had grown tobacco, timothy, corn, or clover during the past year. There seemed to be a slight variation in the quantity of soluble aluminum present (table 2), but there was no apparent relationship between the amount of aluminum found in the soils and the occurrence of the root disease.

TABLE 2.—*Parts per million of aluminum in and pH of soils of the same type upon which various crops were grown during the same year*

Crop grown	Aluminum of soil, soluble in water	Aluminum of soil, soluble in 1 percent citric acid	pH value of soil	Crop grown	Aluminum of soil, soluble in water	Aluminum of soil, soluble in 1 percent citric acid	pH value of soil
Corn	0	0.2	5.95	Timothy	0.3	4.0	5.95
Tobacco	4.0	4.5	5.60	Clover	.2	.3	5.43

EFFECT OF ALUMINUM ON TOBACCO GROWN IN POTS IN THE GREENHOUSE

The analysis of the soil for aluminum after 2½ months of crop growth indicated that nearly all of the aluminum had been converted from a soluble to an insoluble form in the soil (table 3). The water extract of the soil given originally 100 parts per million of aluminum contained at the time of analysis slightly more than 1 part per million. Phosphate added to this combination formed insoluble aluminum compounds that retarded the formation of ionic aluminum.

A sample of soil in a pot of 1-gallon capacity which had grown tobacco affected by brown root rot was analyzed for aluminum. The pH value of the soil was 5.51. The soil was extracted with a 1-percent acid solution and was found to contain 0.9 part per million of aluminum.

As a result of these analyses the writer finds no relation between the aluminum content of soil solution and the occurrence of brown root rot of tobacco. Tobacco plants grown on the soil to which aluminum sulphate had been added to the extent of 100 parts per million of aluminum showed no deleterious effects. In fact, in some respects the growth was more luxuriant than that of the control plants grown in soil to which no aluminum was added.

TABLE 3.—*Parts per million of aluminum in and pH of soils treated with aluminum sulphate and with phosphate, and analyzed after 2½ months of crop growth*

Soil no.	Treatment	Aluminum in soil, soluble in water	pH value of soil
1	Aluminum sulphate equivalent to 100 p. p. m. of aluminum	1.5	4.40
2	Aluminum sulphate equivalent to 50 p. p. m. of aluminum	.3	4.56
3	Aluminum sulphate equivalent to 25 p. p. m. of aluminum	.2	4.86
4	Aluminum sulphate equivalent to 100 p. p. m. of aluminum, and 2.9 g CaH_2PO_4 per 8 pounds of soil	.3	4.55
5	Aluminum sulphate equivalent to 50 p. p. m. of aluminum, and 1.45 g CaH_2PO_4 per 8 pounds of soil	.1	5.00
6	Aluminum sulphate equivalent to 25 p. p. m. of aluminum, and 0.73 g CaH_2PO_4 per 8 pounds of soil	.2	5.05
7	Nothing added	.2	5.05

SUMMARY

Tobacco plants were grown alternately 2 days in a complete nutrient solution and 2 days in solutions containing different percentage proportions of 0.006 M aluminum citrate. Definite toxic symptoms were observed at low concentrations of aluminum. No perceptible increase in rate of growth was observed at low concentrations of aluminum salt.

The presence of phosphates lowers the amounts of ionic aluminum in soil.

No results were obtained that justify the assumption that certain crops give rise to active aluminum when the same soil type is used for their culture.

Tobacco plants were grown in complete nutrient solutions in which the proportions of calcium nitrate and aluminum citrate were varied. The results indicate that the calcium ion may function to some extent to reduce the toxic effects of aluminum. In this group of plants low concentrations of aluminum seemed to increase the growth rate slightly.

DISTRIBUTION AND EFFECT OF PETROLEUM OILS AND KEROSENES IN POTATO, CUCUMBER, TURNIP, BARLEY, AND ONION [1]

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INTRODUCTION

Petroleum-oil sprays and fogs are used increasingly to control insect pests of economic plants. These oils are useful in controlling the aphid vectors of potato virus, but it is first necessary to learn which oils are tolerated by potato leaves. Hence, undiluted oils and cresoap emulsions of petroleum oils were tested on potato leaves to determine: (1) The rates of oil penetration into leaves; (2) the symptoms caused by the oils; (3) the oils and their concentrations that potato leaves tolerate without necrosis; and (4) a rapid, easy, accurate method for predicting the probable injury that an oil spray will cause in potato leaves. The purpose of these and other experiments was to discover the distribution and effect of petroleum oils in species of herbaceous plants treated with sprays or undiluted lubricating oils or kerosenes.

REVIEW OF LITERATURE

Besides oil-spray emulsions, there is increasing use of oils dispersed as fogs in air for controlling insects on fruit and vegetables (Herbert (*4*, *5*) and Parker (*7*, *8*)).[3] Fungicides, and insecticides other than lead and arsenic, are used with the oils, and this avoids costly troubles with spray residues. Ginsburg (*2*) described the effects of oils on greenhouse plants, and cited the work of Allen on the use of oils to control potato leaf hoppers. Rohrbaugh (*9*), illustrated oil penetration in citrus. Knight and Cleveland (*6*) gave evidence that surface tension and viscosity determine the rate of oil penetration. Chandler, Flint, and Huber (*1*) reported safe use of 1 percent oil sprays on potato leaves. Young (*10*, *11*) mentioned symptoms of oil injury in potato leaves, and the effects of oils on potato yields; (*12*) described the use of *Rhizopus* growing in oils to test the toxicity of oils; (*13*) illustrated freezing phenomena in emulsions of spray oils; (*14*) described penetration, distribution, and effects of oils in apple leaves; and (*15*) explained theoretically how oil penetrates protoplasm. Young [4] described physical phenomena of emulsification and (*16*) also the conduction of decane from onion leaves to the roots. Young and Morris (*17*) tested an apple, 22 of the 36 oils described in the present paper.

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[2] The writer wishes to thank Profs. H. E. Morris and D. B. Swingle of the botany department of Montana State College, for criticizing this manuscript.

[3] Reference is made by number (italic) to Literature Cited, p. 933.

[4] YOUNG, P. A. MICROSCOPICAL OBSERVATIONS ON FORMING AND BREAKING CRESOAP EMULSIONS OF PETROLEUM OILS. Abstract of Papers, Amer. Soc. Plant Physiol., Pittsburgh, Pa., p. 17., December 1934. [Mimeographed.] (Illustrated article in press.)

MATERIALS AND METHODS

The 36 kinds of petroleum oils and kerosenes described in table 1 ranged in viscosity from 31 to 410 seconds, and had sulphonatable residues ranging from 0 to 53 percent. These oils were tested on vegetables from 1928 to 1933, inclusive.

TABLE 1.—*Sulphonatable residues of, and injuries to potato leaves by, the oils used*

Oil or kerosene no.	Name of oil or kerosene [1]	Injury factor [2]	Sulphonatable residue [3]	Viscosity	Plants [4]
			Percent	*Seconds*	*Number*
1	St. 13604 R.100P	3.4	25	100	4
2	St. 13605 R.CRE	3.3	44	220	4
3	St. O. T. 13606 R	1.5	5	100	16
4	St. M. S. 13607 R	1.8	10	50	12
5	St. 5, 14510 R	3.6	41.6	110	4
6	St. Diamond paraffin	3.2	High	99	4
7	St. Atlantic Red	3.4	High	244	4
8	St. 410 red engine	3.8	53	410	5
11	St. gas oil	3.9	40	38	9
12	Shell Brown neutral	3.4	27	115	9
13	Shell RL99, 70P	3.7	31.2	75	12
14	Shell 1	3.9	28.5	63	4
15	Shell 2	3.4	26.4	58	5
16	Shell 3	2.4	10	55	15
17	Shell 4	3.4	28.3	108	3
18	Shell 5	2.4	9.8	108	4
19	Shell 6	3.7	34	219	4
20	Shell 105	1.2	2	90	5
21	Shell 106 (SO_2)	3.2	15.6	53	3
22	Shell 107	1.2	2	51	10
24	N. Amalie 10680P	1	0	67	29
25	St. 6, 14819R	3.0	10	73	3
27	Shell 198	3.2	20	60	3
28	Shell 7, 14776R	3.8	43	100	8
31	Stanco Nujol	1.2	0	220	6
33	Shell E512 (SO_2)	2.9	12.8	52	5
34	Shell E513	2.7	13.3	51	5
35	Shell E514 (SO_2)	3.2	13.7	74	5
36	Shell E515	2.5	14	75	5
37	Shell E516	1.7	2.3	55	5
41	St. kerosene 20877	1.7	4	31	8
42	St. kerosene 20878	3.7	19	31	8
43	St. kerosene 20879	3.8	22	31	8
44	Shell kerosene	2.5	1	31	9
45	——do	2.4	4.1	31	9
46	——do	3.7	16	31	9

[1] Company name and number of oils and kerosenes. Symbols signify: L. Sonneborn Sons, Inc., New York; Stanco, Stanco, Inc.; St., Standard Oil Co. (Calif.); Shell, Shell Oil Co.

[2] 1=Necrosis only in heavily oiled spots within 11 days. 2=Necrosis of heavily oiled spots in 1 day and of many lightly oiled spots in 11 days. 3=Necrosis and wilting of heavily oiled spots within 1.5 to 7 hours.

[3] Percentage of sulphonatable residue in each oil as determined by the oil companies, except oils 5, 13, 15, 16, and 21 by Green (*3*).

[4] Number of potato plants on the leaves of which sufficient oil was placed to make 20 to 60 percent of the laminae translucent.

The effect of oil on the yield of potatoes was tested by spraying cresoap emulsions of oil 4 on potato leaves in a field (table 2). The leaves were drenched with the oils on July 9 and 24, 1931. The potatoes were grown in alternating units of 6 sprayed and 6 unsprayed hills. Only one seed piece was planted in each hill. The hills were 20 inches apart in rows 36 inches apart, so there were 8,712 hills per acre. Each yield was calculated in bushels per acre by multiplying the total yield in pounds by the constant, 145.2, and dividing this product by the number of plants producing the total yield.

The oils were emulsified in water with ammonia-casein and cresoap emulsifiers as described by Young and Morris (*17*). The cresoap was made by boiling the cresols and soaps together during 3 days.

Oils 3, 4, 13, 21, 22, 24, 36, and 37 in concentrations of 1, 2, 4, 8, and 16 percent emulsified with cresoap or ammonia-casein, were sprayed on leaves of potatoes growing in an experimental plot. The oils described in table 1 were placed in undiluted form on the leaves of Triumph, Russet Burbank, Irish Cobbler, and Idaho Rural potatoes in a greenhouse and an experimental plot.

Oil 3 was placed on the cotyledons of squash and cucumber seedlings, and oil 24 on the leaves of rutabaga and turnip. Seedlings of barley treated with oils 3, 13, 21, and 24, were supplied by Green (*3*).

Onions also were used in studying oil distribution. Bulbs of Yellow Danvers, Bermuda Yellow, and Spanish Yellow onions were set with their bases in water until they produced new roots and leaves. The leaves, bulbs, and roots were treated with oils 3 and 24. Normal onions were studied for comparison. Oil 24 was injected hypodermically into hollow leaves of onions growing in root-study boxes.

Free-hand sections of fresh, living tissues mounted in distilled water were studied microscopically to determine the distribution and conducting channels of oils in plants. Some samples of oils 3, 13, 16, and 24 had nearly 0.2 percent oil Red O dissolved in them to make the oils easily visible in tissues. Sections containing unstained oils were stained with Sudan IV (*14*).

EXPERIMENTAL DATA

EFFECTS OF OIL ON POTATO YIELDS

The potatoes sprayed with a Mineral Seal oil 4 in a concentration of 1 percent yielded slightly more than the unsprayed potatoes growing in alternate units (table 2). In contrast, the emulsion of 2-percent oil reduced the yield of the potatoes 9.3 percent (table 2). This decrease in yield probably is not very significant when it is compared with the increase of 4.6 percent in the yield of the potatoes sprayed with the emulsion of 1-percent oil, while growing in the same plot. Hence a spray of 1 percent of oil 4 probably has commercial value because it is expected to control aphids without decreasing the yield of the potatoes. A spray with 2 percent of oil 4 is not recommended because it may cause some injury to potato leaves.

TABLE 2.—*Effects of sprays with cresoap emulsions of oil 4 on yields of Bliss Triumph potatoes at Bozeman, Mont., 1931*

Treatment of potato plants	Plants	Yield of tubers	Calculated yield per acre	Increase (+) or reduction (−) in yield as affected by spray
	Number	*Pounds*	*Bushels*	*Percent*
1-percent oil emulsion	55	48.76	128.7	+4.6
Unsprayed	58	49.13	123.0	
2-percent oil emulsion	103	98.26	138.5	−9.3
Unsprayed	109	114.64	152.7	

OIL PENETRATION INTO POTATO LEAFLETS

Hypophyllous applications of the oils to normal Bliss Triumph potato leaves at 20° to 25° C. revealed the following rates of penetration causing immediate translucence: 0.5 to 10 seconds for the kerosenes with viscosities near 31 seconds; 1 to 30 seconds for the oils with viscosities of 50 to 108 seconds; 60 seconds for the oils with viscosities near 220 seconds; and nearly 5 minutes for oil 8 with a viscosity of 410 seconds. The red-stained oils made potato leaves and their stems red during 1 or more weeks. Emulsions containing oils in concentrations of 4, 8, and 16 percent also made oily translucent spots in potato leaves. Similar reactions were observed when oil penetrated the leaves of turnip, rutabaga, onion, and barley, and the cotyledons of cucumber and squash.

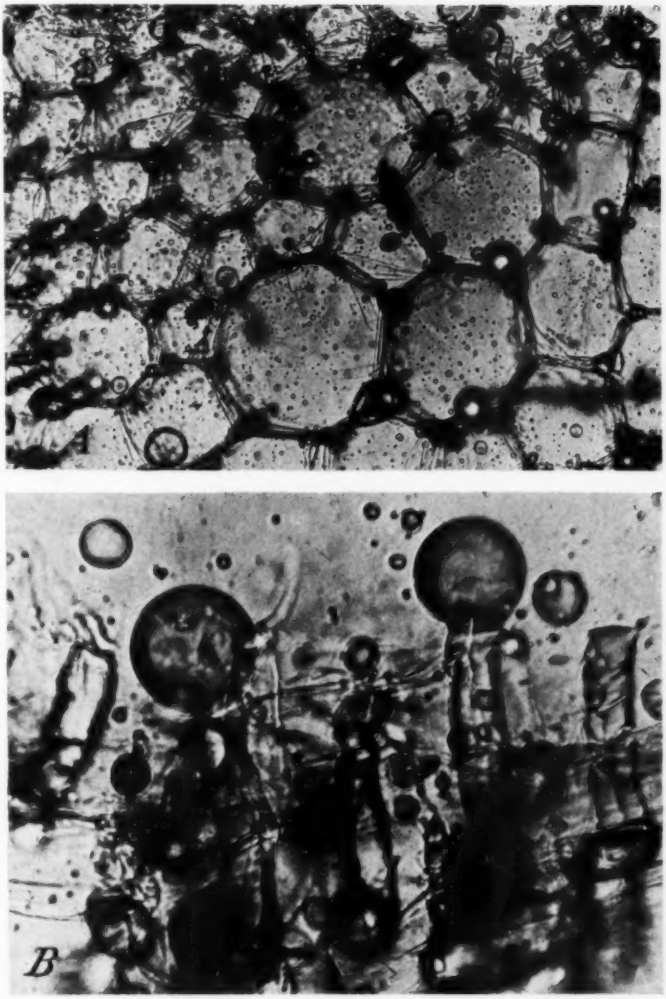
The oily spots in potato and apple leaves were similar in their refraction and reflection of light (*14*). Potato leaves showing oily spots were stored in the herbarium, where many of the spots retained their oily appearance in the dried leaves during 1 or more years, thus resembling apple leaves in their retention of oil.

DISTRIBUTION OF OILS IN POTATO, CUCUMBER, TURNIP, BARLEY, AND ONION

Oils 3 and 24, with and without Oil Red O stain, were placed on the leaves of potato, turnip, rutabaga, and barley, and on the cotyledons of cucumber and squash seedlings. They were also injected hypodermically into the hollow leaves of onions. The stems attached to the oiled leaves usually became oily 1 to 5 days after the leaves or cotyledons were oiled. The red-stained oils made the tissues red. Sections of such plants were cut to determine the distribution of the oils. The stained and unstained oils were similar in their distribution and effect. Examples of sections showing typical distribution of oils are given in table 3. The oils were abundant between parenchyma cells (pl. 1). Less oil was seen in tracheae and parenchyma cells, so the oil evidently passed mostly between parenchyma cells in being carried from the leaves into the stems and roots of these herbaceous plants. The pneumatic and hydrostatic system of the plant is concerned with the conduction of oil. Oil penetration into parenchyma cells was explained by Young (*15*).

Red-stained oil 24 passed from Irish Cobbler potato leaves into the new tubers in the soil, making prominent red regions within 1 cm from the point of attachment of the stolons. By forcing a wire through the pith, vertical holes 0.3 cm in diameter and 12 cm deep were made in 4 large stems of a Russet Burbank potato plant, and 1 cc of oil 24 saturated with oil Red O was placed in each hole. The oil was found in 3 of the 9 attached tubers, 8 days later. The petroleum oil in potato tubers was mainly between the starch-parenchyma cells.

Stems were placed in emulsions to study absorption of oils from emulsions, and to determine the toxicity of emulsion cream. Excised potato stems bearing leaves were stood in jars containing cresoap emulsions of 1 and 4 percent oil 24 with oil Red O. Parts 1 to 2 cm long wilted where these stems passed through the cream layers 1 mm thick. In contrast, the leaves and the parts of the stems above and below the cream layers remained alive for a few days. Flowering peduncles of *Oxalis cernua* Thunb. were stood in cresoap emulsions of 1, 2, 4, and 8 percent concentrations of oils 16 and 24 (with and with-



A, Cross section of the seeding stem of a White Spine cucumber showing the globules of oil (photographed black) above the intercellular spaces of the parenchyma cells. The oil had exuded from the polyhedral tubes between the parenchyma cells. Oil 3 stained with Sudan III was placed on the lower sides of the cotyledons of this cucumber on December 3, 1929. The attached stem was frozen in ice and cut 15 days later. × 160. *B*, Like *A*, but an oblique section of this cucumber stem showing the polyhedral, interparenchyma tubes bearing globules of oil 3 that exuded from them when they were cut. × 633.

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out oil Red O). The parts of the oxalis peduncles passing through the cream layers wilted within 1 to 2 days, while the flowers and the parts of the peduncles below and above the cream layers remained apparently normal during a few days. In contrast, oxalis peduncles standing in water alone, and in water bearing a layer of oil 3 with oil Red O, showed no wilting for several days. Onion leaves were stood in jars containing cresoap emulsions of 1 and 2 percent oil 24 with oil Red O. The parts of the leaves near the cream layers wilted within 9 to 24 hours, while above the cream layers, the parts of the leaves remained turgid.

TABLE 3.—*Distribution of oils in tissues after oils were placed on the leaves of potato, turnip, rutabaga, onion, and barley, and on the cotyledons of cucumber and squash*

Plant	Oil no.	Tissue sectioned	Period between oiling and sectioning	Location of oil [1]		
				Between parenchyma cells	Inside parenchyma cells	Inside tracheae
			Days			
Potato	24	Mesophyll	12	+	0	0
	3	Midrib	23	0	0	+
	3	Petiole	23	+	+	0
	3	....do	11	+	0	0
	24	Stem	55	+	+	+
	24	....do	27	+	+	+
	24	Aerial tuber	39	+	0	+
	3	Roots	11	+	+	+
	3	....do	23	+	+	0
	24	Old seed tuber	38	+	+	0
	3	Stolon	11	+	+	0
	24	New tubers in soil	38	+	+	0
	24	....do	73	+	0	0
Cucumber	3	Stem	14	+	0	0
	3	....do	7	+	+	0
	3	Tap root	7	+	+	0
	3	Fibrous roots	16	+	0	0
Squash	3	Stem	14	+	0	+
Turnip	24	Petiole	23	+	0	0
	24	Root	8	+	0	+
Rutabaga	24	....do	9	+	0	0
	24	Petiole	9	+	0	+
Onion	24	Leaf base	2	+	0	0
	24	Root	2	+	0	+
	24	....do	20	+	0	+
Barley	21	Leaves	5	+	+	+
	3	Stems	4	+	0	0
	13	Roots	5	+	0	+

[1] +=oil present; 0=oil absent.

Oil 24 was only slightly toxic while cresoap was very toxic (Young and Morris, *16*). Stems wilted where they passed through layers of emulsion cream, which is evidence that abundant cresoap in the cream penetrated the stems. Although wilting occurred in the parts of stems touching cream, the tracheae in the wilted parts continued to conduct water, so the tops of the stems remained alive and turgid for a few days.

Oil 3 saturated with Sudan III was placed on the cotyledons of seedling cucumbers (*Cucumis sativus* L.) variety White Spine, and seedling squashes (*Cucurbita maxima* Duch.). The oil made the cucumber and squash cotyledons red and oily translucent within 10 seconds. The attached stems also became red and oily translucent within 1 to 14 days. Sections of these stems showed the oil mainly between the

cortical parenchyma cells (pl. 1; table 3). These sections containing oil prominently showed the concave-sided polyhedral tubes between the cortical parenchyma cells.

Sections of cucumber and squash stems not attached to oiled cotyledons showed air bubbles in the polyhedral spaces between the parenchyma cells. No oil was seen in these stems.

Oil 24 with oil Red O made leaves of turnip (*Brassica rapa* L.) and rutabaga (*Brassica napobrassica* Mill.) red and oily translucent. The attached petioles and stems also became red and oily within 1 to 8 days. The attached fleshy turnip and rutabaga taproots were sliced 8 days after the leaves were oiled. Large parts of the tissues were red because of the red oil in them, in contrast to the nonoily parts of the same roots (pl. 2, *A*). Microscopic study of thin sections of stems and roots revealed the oil between the parenchyma cells (table 3).

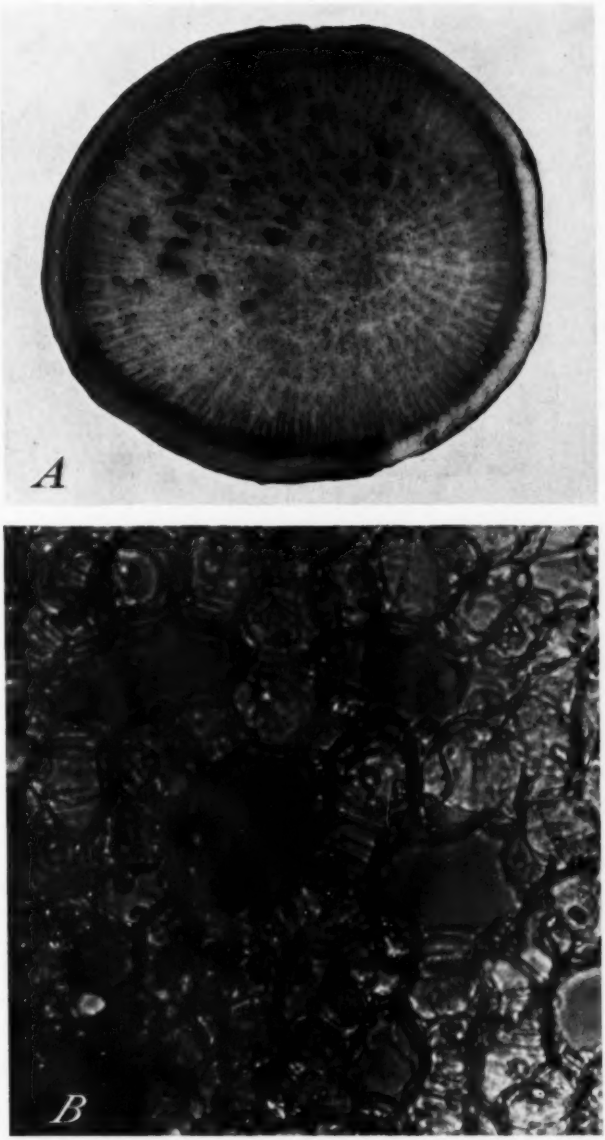
Leaves of barley seedlings (*Hordeum vulgare* L.) turned red and oily translucent within a few seconds after the red-stained oils 3, 13, and 24 were placed on the leaves. The attached stems and roots also turned red within 4 days. Sections of these stems and roots showed oil between and inside the cells (table 2). Oil was seen between collenchyma cells in a stem. Oil 21 was placed on seedling leaves of barley. Sections of the oiled leaves were cut 5 days later and were stained with Sudan IV. The oil was abundant between the chlorenchyma cells, and in the tracheae of a large vein.

Five bulbs of onion (*Allium cepa* L.) were set in root-study boxes. When the new leaves were 5 to 25 cm long, 1 to 4 cc of red-stained oil 24 were injected hypodermically into the tip of each of 1 to 4 hollow leaves of each bulb. During 3 to 9 days, these hollow leaves were oil reservoirs that exhibited the columns of oil by transmitted light. Thirty to ninety percent of the attached roots turned red within 2 to 10 days after the oil was injected into the leaves. Sections of the roots and bulbs showed the oil in their tissues (table 3 pl. 2, *B* and 3).

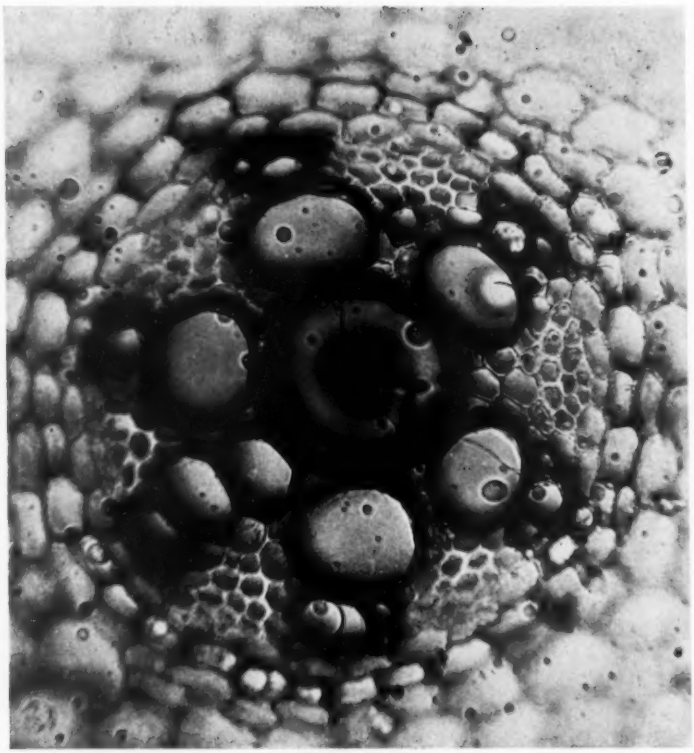
Oil 24 saturated with indophenol blue was injected into the hollow leaves of four other onion bulbs. The attached roots did not change color so some of them were sectioned and stained with Sudan IV, which revealed the oil in and between the cortical parenchyma cells. Indophenol blue greatly increased the toxicity of oil 24 in contrast to oil Red O that apparently did not increase the toxicity of this oil.

The roots of three onion bulbs were placed in jars of water bearing layers of oil 24 with oil Red O. The oil entered many of these roots and was distributed in the attached leaves and bulbs. Two onions were set so that the bulbs were 6 cm above the water into which their roots projected. Oil 24 with oil Red O was placed on the aerial parts of their roots. The oil entered many of these roots and was conducted into the bulbs.

Entrance of oil through wounds was studied by excising the roots on onion bulbs and setting the bulbs in shallow layers of oils 3 or 24 with oil Red O. The bulbs became red within 20 hours. Sections showed oil in the tracheae of a flattened bulb-stem, because oil entered through the cut base of this stem. Also, excised onion leaves remained alive and turgid during 1 to 3 days while they stood with their cut ends in oil 24 with oil Red O. Much oil entered these leaves. No oil was seen in any of the check onions studied for comparison with the onions treated with oils.



A, Oil 24 stained with oil Red O (photographed black) in a cross section of a rutabaga root 8 days after this oil had been placed on the attached leaves; much of the oil had passed from the leaves into the fleshy root. × 1. *B*, Oil 5 stained with oil Red O (photographed black) occupying the vertical interparenchyma pockets in the solid part of a second-year onion stem inside the bulb. This cross section was cut after the base of the bulb and the upper parts of the roots had touched the oil during 11 days. × 296.



Oil 24 stained with oil Red O (photographed black) in the central trachea of a root of a Sweet Spanish onion The oil in this root came from one attached hollow leaf into which 3.5 cc of oil were injected hypodermically 20 days previously; 13 of the roots became red within 2 days. × 305.

The roots of six onion bulbs were arranged so that half of the roots on each bulb projected into water alone, while the other roots projected into test tubes of water bearing layers 1 cm thick of oils 3 or 24 with oil Red O. The oil entered the roots that had wounds in their parts in the oil layers. Branch roots naturally make wounds as they rupture their way through the cortex. The sides of the bulbs turned red above the oiled roots. Also some of the other roots turned red within 3 days, although they projected into water alone. Sections cut 2 days later showed oil in the tracheae and parenchyma cells of these roots. A small aerial leaf on one bulb turned red. Sections of it showed oil between the chlorenchyma cells. Thus, oil was conducted from the roots into this leaf. Sections of the red parts of these bulbs showed oil between the parenchyma cells of the fleshy bulb scales (bases of first-year leaves). Also the cylindrical stems in the bulbs (bases of the second-year leaves) showed abundant oil between the parenchyma cells, especially inside the vertical interparenchyma gas pockets (pl. 2, *B*).

Oil 24 with oil Red O facilitated determination of the nature and function of the large interparenchyma pockets (pl. 2, *B*). These apparently vertical pockets were surrounded by specialized parenchyma cells. The interparenchyma pockets abounded in the leaves and in the cylindrical stems inside and above the bulbs. Sections showed that these pockets naturally contained gas, but were filled with oil in many of the onions treated with oils. These pockets were not seen in very small leaves and their stems inside onion bulbs, but developed as the leaves and stems matured. None of these gas pockets was seen in the fleshy bulb scales, nor in the flattened bulb stems. These flattened bulb stems showed parenchyma cells with ramifying tracheae that usually did not contain petroleum oil. The interparenchyma gas pockets that were centrally situated in maturing onion leaves showed ruptured walls. Evidently many of the pockets united to form the hollow centers of the maturing leaves. These interparenchyma pockets are part of the hollow mechanical structure of onion leaves.

SYMPTOMS CAUSED BY LUBRICATING OILS AND KEROSENES IN POTATO LEAFLETS

Undiluted lubricating oils placed on potato leaflets often caused wilting preceding necrosis in oily translucent leaflets. Some of the oiled juvenile leaflets rolled upward. They were more susceptible to oil injury than were mature leaflets. Sprays of lubricating petroleum oils in concentrations of 4, 8, 16, and 100 percent commonly caused black and brown spots in potato leaflets. Oils often accumulated over epiphyllous veins and blackened them. Large amounts of toxic oils killed the leaves and stems. Sprays with emulsions containing 2 percent of toxic oils often caused brown spots 1 to 10 mm wide in leaflets.

Oil 21 caused purpling in some leaflets. Probably the other oils lacked sufficient concentration of the chemicals that caused purpling in potato leaflets.

Epiphyllous white and light-gray spots appeared in many potato leaflets treated with 4-, 8-, and 100-percent concentrations of oils 4, 7, 13, 21, 27, 42, 43, and 45. Most of these white spots were hypophyllously green, brown, or dark gray. Epiphyllous white spots served as a useful symptom in distinguishing these oils. Probably

only these oils contained the casual chemicals in concentrations sufficient to cause white leaf spots. Oils 13 and 21 caused white spots abundantly and regularly, so that prominent production of this symptom distinguished oils too toxic for use in sprays on potato leaves.

The translucence caused by kerosenes usually disappeared within 1 to 24 hours, as the kerosenes evaporated from the intercellular spaces. The kerosenes caused chlorosis, brown spotting, marginal rolling, and blackening and drying of the rolled parts. The petioles died on many of the badly injured leaves.

Oils 41, 44, and 45 caused no injuries or only mild injuries in potato leaves, while oils 42, 43, and 46 caused wilting within 2 hours in heavily oiled leaflets, and killed many of the petioles (table 1).

INJURIES CAUSED BY PETROLEUM OILS IN POTATO LEAVES

In table 1, the physical suffocating effect of the oils presumably caused injuries represented by the injury factor 1, as oil 24 did, whereas additional injuries represented by larger injury factors such as 1.2 to 3.9 presumably include the chemical effects of toxic compounds in the oils, according to a critical definition of toxicity (*14*). Oils 14, 15, 44, and 46 were more injurious than their percentages of sulphonatable residues indicated, probably because some of the sulphonatable chemicals were especially toxic to potato leaves. Usually, the kerosenes and lubricating oils with low percentages of sulphonatable residues caused only slight injuries, even when they made the laminae translucent. In contrast, the more sulphonatable oils caused serious necrotic symptoms even when they were applied in concentrations of only 2 percent.

Practical conclusions are drawn from table 1. The toxic effects of oils in potato leaves were tested accurately, easily, and quickly by placing drops of each undiluted oil on normal potato leaves at temperatures below 35° C., and before the leaves show senescence. The rapidity, severity, and abundance with which the symptoms appeared showed the toxicity of each oil to potato leaves. The oils that caused amounts of injury approximately indicated by the injury factor 3 presumably are too injurious for sprays on potato leaves. The oils that caused amounts of injuries indicated by the injury factor 2 probably are too toxic to use in emulsions containing more than 1 percent of oil. The oils that caused amounts of injury indicated by the injury factor 1 are preferable for sprays in concentrations of 1 and 2 percent of oil on potato leaves.

SUMMARY

Cresoap emulsion of 1-percent oil 4 did not reduce the yield of potatoes, while an emulsion of 2-percent oil caused a 9.3 percent reduction in the yield.

Lubricating oils caused the following symptoms in potato leaflets: Translucence, wilting, rolling, chlorosis, blackening and browning of veins, and spots of laminae; also purpling and white leaf spots. Only the very toxic oils caused severe injuries including purpling and white leaf spots.

Kerosenes caused the following symptoms in potato leaves: Temporary translucence, chlorosis, brown leaf spots, marginal rolling with

blackening of the rolled parts, and killing of petioles. Only the very toxic kerosenes caused the severe symptoms.

A convenient method of testing the toxicity of oils and kerosenes before they are sprayed on potato leaves is to place drops of each oil, and drops of unsulphonatable oil, on healthy potato leaves, and observe the comparative symptoms. Oils that cause more than a few necrotic symptoms within the first few days probably are too toxic for commercial sprays on potato leaves.

The oils and kerosenes with low percentages of sulphonatable residues usually caused only slight injuries even when the laminae had been oily-translucent. The slightly toxic oils may be used safely in sprays of 1 percent oil on potato leaves.

Kerosenes penetrated potato leaves within 0.5 to 10 seconds, but mostly evaporated from the leaves within 1 to 24 hours. In contrast, lubricating oils penetrated potato leaves within 1 second to 5 minutes, and usually remained in the leaves during the remainder of the growing season. Sections showed that the oils passed from potato leaves through the stems and into the tubers.

Emulsion cream caused wilting in stems of potato and oxalis, but did not quickly stop the conduction of water through the wilted parts of the stems.

Red-stained oils placed on cucumber and squash cotyledons quickly passed into the stems and made them red. Sections showed the oil mainly between the cells.

The red-stained oils placed on turnip and rutabaga leaves passed into the fleshy tap roots and made large parts of them red. When red-stained oils were placed on barley leaves, they passed through the stems and made the roots red. The oil Red O stain apparently did not affect the toxicity of either oil 3 or 24.

Red-stained oils injected into onion leaves passed through the bulbs and made many of the roots red. Sections showed the oil in the tracheae and between the cortical parenchyma cells. Oils also passed from roots into attached bulbs and leaves. Leaf and stem sections showed oil in the large pockets between the cells. These interparenchyma pockets in leaves, and second-year stems in bulbs, apparently serve as gas chambers.

Study of these vegetables showed that the petroleum oils were mainly between the parenchyma cells, but also were inside tracheae and parenchyma cells. Evidently, the oils are conducted mainly between the cells in passing from leaves into roots.

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THE DEVELOPMENT OF THE COTTON EMBRYO[1]

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INTRODUCTION

The development of the cotton embryo was treated briefly from the morphological point of view by Balls (*1*),[2] Flatters (*2*), and Gore (*4*). None of these investigators, however, made a chemical study of the developing embryo. Ordinarily such investigations have not been made because of the impracticability of separating the immature embryos from the surrounding tissues in sufficient quantities to make satisfactory analyses. Instead of the usual chemical analyses it is often practical to use microchemical tests, for, although they have the disadvantage of not yielding quantitative results, they have the advantage of being applicable to plant tissues in their natural position and to comparatively small quantities of material.

This study of the development of the cotton embryo was undertaken for the purpose of obtaining a knowledge of the rate of growth, anatomical development, and chemical development, as related to each other and to the age of the embryo from the first division of the zygote to dormancy.

METHODS

In July 1934 approximately 150 flowers of an American upland cotton (Startex) were self-pollinated. As the time after pollination that is required for fertilization to occur has been reported by Gore (*4*) to be from 26 to 32 hours, the present report includes no studies to determine the length of this period, but it is assumed that fertilization occurred within 36 hours. Material was collected, killed in Licent's fluid, sectioned, and stained with haematoxylin or safranine. Collections of embryos were made at the following periods (days) after fertilization, allowing 36 hours for fertilization to be completed: 1, 2, 3, 4, 6, 9, 12, 15, 16, 18, 22, and 26. Before the twenty-sixth day most of the important anatomical developments had occurred, and the oldest of the material was not used for anatomical studies. However, microchemical tests and growth studies were made on the older material.

Some difficulty was experienced in making microchemical studies on fresh material before the sixth day. This was caused by the minuteness of the embryo and the fact that it gave the same reactions as the endosperm surrounding it.

Weights of embryos 16 days of age and older were taken immediately after their dissection from the ovules, and again after drying in air for 24 hours. The lengths of embryos were recorded from the sixth day until maturity.

[1] Received for publication July 22, 1935; issued February 1936. Contribution from the Division of Agronomy, Texas Agricultural Experiment Station, as Technical Paper No. 333.

[2] Reference is made by number (italic) to Literature Cited, p. 944.

EARLY DEVELOPMENT OF THE EMBRYO

The first division of the zygote, which ordinarily occurred the second day after fertilization, was usually horizontal (fig. 1, *A*). This was followed by a vertical division of the apical and sometimes of the basal cell (fig. 1, *B*). Occasionally a diagonal division instead of the vertical occurred in the apical cell, giving rise to such a structure as that shown in figure 1, *C*. The exact manner of origin of this configuration was not determined because of its relatively rare occurrence. A second departure from the condition shown in figure 1, *B*, occurred when the basal cell failed to divide (fig. 1, *C*, *D*), resulting in a one-celled suspensor. Three-celled proembryos were most frequently found in ovules killed the second day after fertilization.

An intermediary tier of cells was found (fig. 1, *K*), but never a single intermediary cell, as was reported in *Malva rotundifolia* by Souèges (*6*). A perfect quadrant was probably formed, and various slight departures from it were common. A group of three cells at the apex (fig. 1, *E*) also was found. The structure shown in figure 1, *E*, occurred in material killed the third day after fertilization.

A short suspensor was formed, but in some cases it showed early signs of disorganization. The latter fact may explain Balls' (1) statement that the cotton embryo has no suspensor.

In material taken the fourth day it was found that octants (fig. 1, *H*) had been produced from the quadrants by further cell division. About the same amount of irregularity was observed in the octants as had been found in the quadrants. Material taken the same day also showed mitoses and periclinal walls separating off the dermatogen (fig. 1, *G* and *I*).

Although the stages found here are irregular, if the succession shown in figure 1, *A*, *B*, *D*, *F*, *H*, and *J* is followed, the history of the development of the cotton embryo will be seen to be similar to that described by Souèges (*6*) in *Malva rotundifolia*. He gave the following account: The first division is horizontal and is followed by a vertical division. The bipartition of the elements of the tetrad gives an octocellular proembryo possessing four circumaxial cells in its upper part. Sometimes 1 of the 2 juxtaposed elements of the tetrad is segmented horizontally or obliquely. The 4 quadrant cells at the apex are separated by walls which take insertion on the peripheral membrane and come down to the vicinity of the axis on the lower horizontal wall (prenant insertion sur la membrane périphérique et venant tomber au voisinage de l'axe sur la paroi horizontale inférieure). Each of the 8 cells then divides, giving rise to a 16-celled proembryo. The wall of segmentation of the quadrant is often horizontal. Five tiers of cells are produced that give rise wholly or in part to cotyledon, hypocotyl and initials of the central cylinder, cortex at the summit of the radial central portion of the tip, and suspensor. This description of the embryogeny of *Malva rotundifolia* appears to be the only record in the literature relative to the early stages of the developing embryo in any malvaceous plant other than cotton.

A great deal of irregularity was found in the details of the early stages of development in the cotton embryo; however, the stages of development illustrated were studied carefully and to all appearances are natural. No doubt the various embryos were sometimes observed from different sides, but if they were radially symmetrical and regular

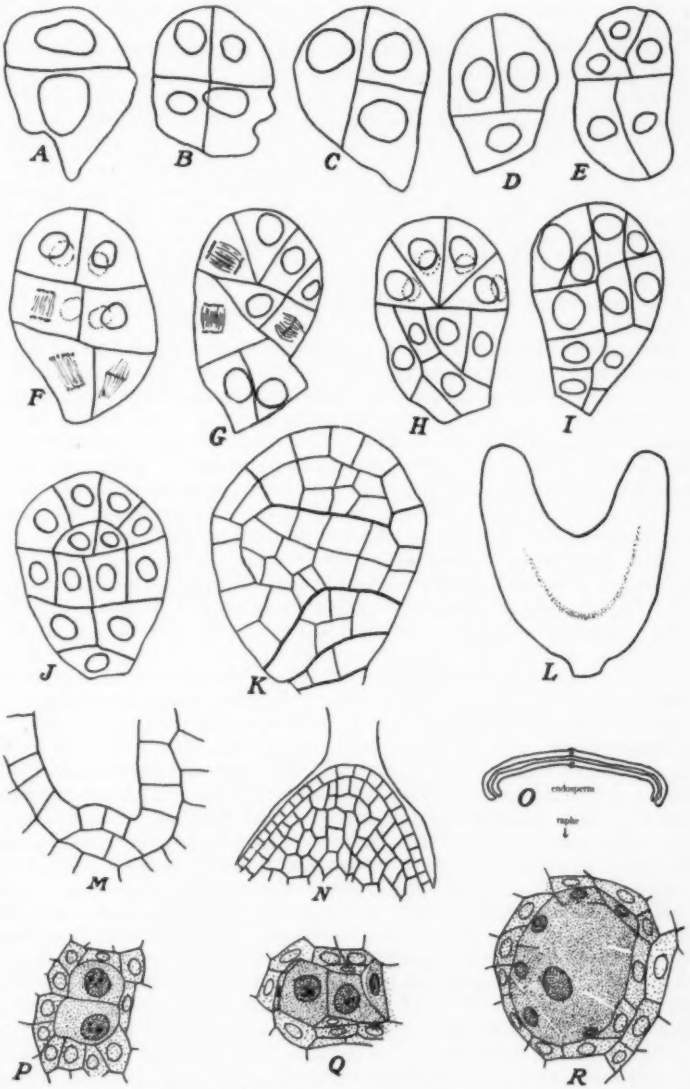

FIGURE 1.—*A–K*, Stages in development of the cotton proembryo and embryo in approximate succession, including irregular conditions, × 550, *L*, 9-day-old embryo showing early differentiation of cotyledons, hypocotyl, and histogens, × 123; *M*, apex of a 9-day-old embryo in a slightly later stage of development, showing the beginning of the plumule, × 550; *N*, plumule of a 15-day-old embryo, × 240; *O*, cross section of cotyledons of a 12-day-old embryo, showing their position with reference to the endosperm and raphe × 20; *P–R*, successive stages in the development of resin glands, × 550.

in early development, as proembryos are often thought to be, their appearance would always be much the same.

Because of the irregularities noted it is impossible to state the exact age at which cotton embryos reach any particular stage of development, but the data given throughout this paper probably represent the conditions that occur most frequently at the ages indicated.

The first microchemical tests were made when the embryo was 6 days of age. At this time, carbohydrates (Molisch reaction) and proteins were present. Negative results were obtained, however, when tests were made for starch, pentosans, and oils. The endosperm, which was very abundant at this age, gave exactly the same reactions as the embryo.

THE GRAND PERIOD OF DEVELOPMENT

The early development of the cotton embryo, as described above, took place before the rapid increase in size of the embryo had begun. About 9 days was required for the initials of the main organs, such as cotyledons, hypocotyl, and plumule, to be formed. The embryo then entered a period of rapid growth, and details of structure continued to manifest themselves as development proceeded. Organization of the cotyledons began between the sixth and ninth day (fig. 1, *L*), when the embryos became heart-shaped in general outline with the two auricles developing into cotyledons and the ventricle end into a hypocotyl.[3] Soon after the cotyledons had assumed their form a small mound of tissue could be observed in the axil between them (fig. 1, *M*). This was the first appearance of the plumule as such. The plumule did not become conspicuous until the fifteenth day (fig. 1, *N*).

Between the sixth and ninth day, the histogens of the hypocotyl made their appearance. The limits of the dermatogen, periblem, and plerome were recognized without difficulty on the ninth day. In material of this age proteins and starch were found to be present and pentosans and oil were absent. Reactions in the endosperm indicated a slight amount of starch, but otherwise the same materials were detected there as in the embryo. At 12 days of age the embryos averaged about 1.6 mm in length, and had taken a position on the side of the embryo sac approximately opposite the raphe (fig. 1, *O*).

In embryos 12 days of age the cotyledons averaged about 7 layers of cells in thickness, these layers being the upper and lower epidermis and 5 layers of mesophyll. The cells were fairly uniform in size and shape and often were in regular rows. As the embryos advanced in age the cotyledons increased in thickness by an increase in size and number of cells. At 12 to 15 days of age the earliest signs of provascular strands were found in the cotyledons. These signs appeared first through the formation of cell walls horizontal to the surfaces of the cotyledon, and later by walls vertical to these surfaces and longitudinal to the axis of the provascular strands. Differentiation of the palisade cells of the cotyledons had begun, also, at this age, the second layer of cells from the upper surface being 50 to 100 percent longer than the other mesophyll cells.

[3] The term "hypocotyl" is used in this paper in the broader sense to include all the organs of the embryo below the attachment of the cotyledons.

In 15-day-old material several other changes were apparent, the most interesting being the first signs of the resin glands. The first indication of a gland was the appearance in the cotyledon of a few (usually 2 or 3) large cells whose protoplasm was very granular and stained rather darkly (fig. 1, *P*). The surrounding cells were of ordinary size and shape except that they were slightly flattened. As the embryo advanced in development, the large cells increased in size, and the cells surrounding them became more flattened (fig. 1, *Q*). Signs of disorganization of the protoplasts of the large cells were obvious on the sixteenth day, for the nuclei had lost their identity, cell walls had begun to disappear, and the contents of the cells had assumed a still more granular, but otherwise homogeneous, appearance. A few layers of the surrounding flat cells also became disorganized and the cells surrounding them in their turn became flat and showed signs of disorganization (fig. 1, *R*). At this age the young glands were well on their way in development, and no structural changes were found between this condition and maturity, except a continuation of the phenomena already described.

Microchemical tests made on embryos 16 days of age showed traces of oil but no pentosans. Reactions of protein with biuret and Millon reagents were more intense in the embryo than in the endosperm, and this difference was observed until the seed was mature.

In embryos 18 days of age, the contents of the young resin glands gave the same color reaction with sulphuric acid that had been obtained in glands of mature embryos. Marchlewski (*5*) attributed this reaction in the mature embryos to the presence of gossypol. This test is by no means specific for gossypol, but when carefully used it is a fair indication of that substance. Sulphuric acid will produce a red color in other parts of the embryo, but the particular shade of color and the streaming of the gland contents observed at this stage of development are identical only with those obtained in the glands of mature embryos. It may well be emphasized here that the reactions indicating gossypol were not observed at the time of the first appearance of the glands, but only in embryos 18 days old or older. Gallup (*3*), in his studies of the time of appearance of gossypol in cottonseeds found gossypol first in 32-day-old seeds. His analyses were made on the entire ovule, rather than on the embryo alone as was done in the present investigation. Naturally, gossypol would be relatively less abundant in the entire ovule, and this may account for his not finding it in the young ovules where it had not become plentiful.

A study of 18-day-old material showed that pentosans had appeared in the glands and that the amount of oil in the cotyledons had greatly increased. Starch and proteins were still abundant in both the embryo and endosperm; and since it is well known that proteins, oil, and pentosans are common in fully mature embryos, tests for them were discontinued at 18 days after fertilization. Starch, however, was known to practically disappear before maturity, and in order to determine the time of its disappearance, tests were made for it until this condition was found. Starch seemed to disappear so gradually that the time when it began to decrease was not determined. It was found to be scarce at 33 days after fertilization; and traces were rarely found in seeds that were apparently mature. When the embryo was 18 days of age, all of the organs and provascular tissues were well on their way in development. All important chemical components of

the embryo identified in this study had also begun to appear, and most of them were fairly abundant. The remainder of the period of development of the embryo must therefore consist chiefly of changes (usually increases) in the amounts of the various components and increases in the size of the organs and in the size of the embryo as a whole.

Microchemical tests for sugar in the embryos were never positive except possibly with the Molisch and the Fehling tests. Tests for sugars were made not only on the embryo but also on young lint hairs and the seed coat. The results of these tests are given in table 1. From this table it may be seen that glucose is present in the developing lint hairs. The results were checked and found identical with results obtained on known corresponding substances. Although tests for glucose, except in the form of glucosides, conducted by other investigators on all parts of the cottonseed have consistently given negative results, there can be no doubt that the results obtained in this study on the young lint hairs are typical glucose reactions. In addition to the results summarized in table 1, tests for glucose were made on lint hairs soon after their appearance and also in the later stages of development. The results showed that glucose is an important component of the lint hairs from the time of their first appearance until the boll begins to open. The occurrence of glucose in these hairs is not surprising in view of the fact that the hairs are composed chiefly of cellulose and that a close relationship is known to exist between glucose and cellulose.

Positive reactions for glucose and fructose were obtained on the seed coat during its development, although these results were somewhat masked by a flocculent precipitate of some other component. After the reactions had been carefully studied and the tests repeated on filtered extracts of the seed coats, it was concluded that this structure contained substances that gave the reactions of fructose and glucose. The glucose reaction was not as strong as that of fructose.

TABLE 1.—*Summarized results of microchemical tests for sugars in the cottonseed coat, embryo, and lint hairs 10 to 26 days after fertilization*

Test	Lint hairs	Seed coat	Embryo
Phenylhydrazine	Positive, glucose	Positive? glucose	Negative.
Flückiger	do	Positive? glucose and fructose	Negative?
Fehling	Positive	Positive	Positive?
Molisch	do	do	Do.

Embryos gave the Molisch reaction for carboyhdrates from their early formation to maturity; however, the Flückiger and phenylhydrazine tests showed that this was not the result of the presence of glucose or fructose. Several other sugars and glucosides are known to occur in mature cotton embryos, and probably one of them was the substance that gave the reaction for carbohydrates. Indeed, starch, which is known to be present in developing embryos, gives positive reactions with the Molisch reagent, but it usually reacts more slowly than the carbohydrate under investigation here. The Fehling test showed a somewhat doubtful reaction for reducing sugars. A precipitate occurred, but its appearance was not typical of the copper oxide that precipitates when the test is applied to any of the common sugars.

THE CLOSING PERIOD OF GROWTH

In the closing period of growth of the embryo there were fewer changes than in the grand period. Storage materials continued to form, so that there was a slow increase in dry weight, but there was a great loss of water and therefore a loss in total weight. No new tissues or organs appeared and no new chemical components were recognized.

THE GROWTH CURVE

For several decades biologists have recognized the fact that the normal growth curve of an organism takes the general form of a somewhat straightened S; that is, growth begins slowly (the formative period), later becomes much more rapid (the grand period), and then again becomes slow preparatory to cessation (the maturation period). In the life of the sporophyte of a cotton plant, there are at least two such growth curves. The first represents the development of the embryo from the zygote to the mature seed in the resting period, and the second represents the time from the awakening of the embryo from its dormant period until the sporophyte has reached maturity. In the cotton embryo, the exact form of the growth curve (figs. 2 and 3) was found to vary in some details, depending upon whether the size of the embryo was recorded as length, immediate weight, or air-dry weight. The curves are very similar, however, and the differences are easily explained. It was not found practical to weigh embryos until 16 days after fertilization, but measurements of length were taken as early as 6 days after fertilization. The embryo entered upon its grand period of development in length about a week before it entered this period of development in weight. This is explained by the fact that during early development length increases more rapidly than volume. On the fifteenth day the embryos were approximately 3.8 mm long. Then they began to expand laterally, filling the cavity of the growing seed coat. From that time until the seed reached its greatest length, the weight and length curves were very similar; however, when this period was reached, about the twenty-sixth day after fertilization, the weights continued to increase at the same rate. The last 3 days of this period make up the period of maturation. The embryos continued to increase in actual size (live weight) until the thirty-fourth or thirty-fifth day, when they began to lose weight by drying. They

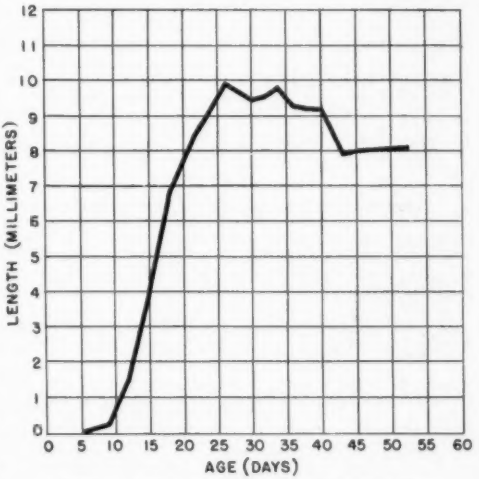

FIGURE 2.—Length of cotton embryos at different ages.

reached an equilibrium between the forty-third and forty-seventh day and then remained constant. The period of maturation in live weight occupied from the thirtieth to the thirty-fourth day. In air-dry weight, the embryos increased steadily until the thirty-second day and then entered upon the period of maturation. They attained their greatest weight on the fortieth day, although the variation observed in the curve after the thirty-sixth day was probably caused by the smallness of the samples. The curves based on weights show the period of maturation more adequately than the curve based on length. Gallup (*3*) states that the weight of the embryo may be expected to decrease slightly after maturity, possibly through respiration. A decrease would naturally occur in any seed, but the amount of decrease would be negligible.

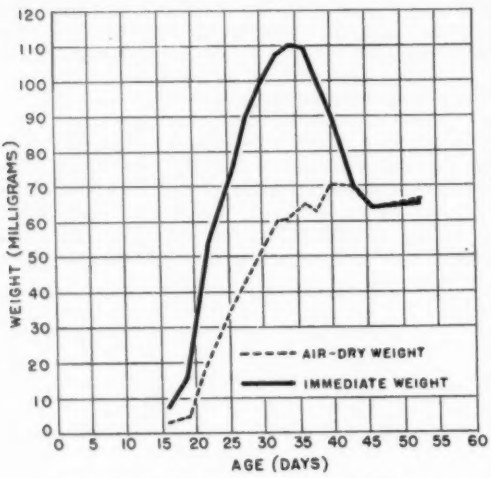

FIGURE 3.—Weight of cotton embryos at different ages.

The principal events observed in the developing embyos with their corresponding ages are summarized in table 2.

TABLE 2.—*Age in days of cotton embryos with corresponding morphological and chemical changes, green weight, dry weight, and length*

Morphological and chemical changes	Age	Embryos weighed	Green weight	Dry weight	Embryos measured	Length
	Days	*Number*	*Mg*	*Mg*	*Number*	*Mm*
Occasionally first division of zygote	1					
First or second division of zygote	2					
Quadrant and other similar stages	3					
Octant; separation of dermatogen	4					
Carbohydrates (Molisch reaction) and proteins present	6				2	0.07
Early organization of cotyledons and hypocotyl; appearance of histogens and starch	9				2	0.29
Location of embryo at side of embryo sac opposite raphe	12				15	1.6
Appearance in cotyledons of provascular strands, resin glands, and palisade tissue; early differentiation of plumule, appearance of oil	15				16	3.8
	16	20	7.5	3.0		
Appearance of gossypol and pentosans	18				13	6.8
	19	14	16.4	4.6		
Continuation of growth and differentiation of tissues that had already arisen	22	14	53.6	21.0	5	8.6
	26	20	77.8	38.0	12	9.9
	30	55	100.2	52.0	30	9.5
	32	14	107.1	60.0	29	9.6
	34	20	110.3	61.2	20	9.8
	36	59	109.8	64.7	31	9.3
	38	76	99.4	63.0	76	9.2
	40	50	90.6	70.2	50	9.2
	43	44	70.2	69.5	44	7.9
	46	30	63.5	63.3	30	8.0
	53	20	65.7	65.9	20	8.1

ABNORMALITIES AND IRREGULARITIES

Although the material used in this study was thought to be a relatively uniform race of American upland cotton, much irregularity was observed in the size of embryos of identical ages.

A few examples will suffice to show the range in size of embryos of the same age. Three 29-day-old bolls were taken at random, embryos were dissected from them, and the average live weights were found to be as follows: Boll 1, 20 embryos averaged 72 mg; boll 2, 20 embryos averaged 111 mg; and boll 3, 15 embryos averaged 123 mg.

After the bolls had been dried in air until an equilibrium was reached, the following average weights were recorded: Boll 1, 20 embryos averaged 33 mg; boll 2, 20 embryos averaged 60 mg; and boll 3, 15 embryos averaged 63 mg. It should be emphasized that these figures are the averages of 15 or 20 embryos, taken at random from the boll, and that the variation among individual seeds was probably much greater than that shown here.

Among the embryos from a single 19-day-old boll the range in length was from 3.5 to 7 mm. If this range were stated in volume or weight it would be much greater. In a fully open, 53-day-old boll, the range in length of 10 embryos taken at random was from 6 to 9 mm. This was an ordinary sized boll having 5 good locks and 37 seeds.

Such cases of variation were not at all uncommon, although it cannot be said that they were of regular occurrence. Whenever so wide a range of variation occurred it was usually in part the result of the fact that a few embryos were much smaller than others. For example, in the last case cited above, the individual measurements in millimeters were as follows: 6, 7.5, 8, 8, 8, 8, 8, 8.5, 8.5, and 9. Numerous ovules of all ages were found which at first appeared to be entirely devoid of embryos, but which upon close examination showed small, sickly ones.

Ovules that showed no embryo sacs, or very abnormal ones, were found occasionally. Undeveloped ovules caused by a lack of fertilization were common, of course, but were not among the irregularities studied. Observations indicated that the position of the seed in the locule was related to its ultimate size. Position is undoubtedly related to shape of embryo and therefore to length. Rea (7) obtained some evidence that motes, or abortive ovules, are caused by a lack of fertilization. Some of the irregularities reported here are undoubtedly related to motes, and further embryological studies should reveal useful information as to how they originate.

SUMMARY

A study was made of the anatomical and chemical development of the cotton embryo in relation to its rate of growth.

The early anatomical development was found to be somewhat irregular, but it showed certain similarities to the early embryonic development of *Malva rotundifolia* as reported by Souèges.

Details of the development of the resin glands were studied. Indications of gossypol were found much earlier in the development of these glands than had been previously reported.

Most of the organs and tissues began their development during the latter part of the formative period and the first part of the grand period of growth. On or before the eighteenth day, oil, starch, pentosans, gossypol, and proteins were formed. All of these materials, except starch, were found throughout the remainder of the growth period.

Glucose was not clearly demonstrated in the embryo at any time during the entire period of development, but it was found in the young lint hairs from the time of their first appearance until just before maturity.

A high degree of variation in rate of growth and in size of embryos within the same boll was found, in spite of the fact that the material was thought to be relatively pure and the flowers had been self-fertilized. Numerous irregularities in form were observed in mature embryos.

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THE CHROMOSOME NUMBER IN GLADIOLUS [1]

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INTRODUCTION

The genus *Gladiolus* is known best by its many summer-flowering varieties. The exact origin of these types in many cases is uncertain, but it is generally agreed (*2, 3, 6, 11, 12, 13*)[2] that interspecific hybridization followed by rigorous selection has played an important part in their development. It appears that such hybrids have often arisen from crosses between very different species and that eventually not only 2, but sometimes 3 and even 4 species have been involved. The high degree of fertility in the commercial varieties, in view of their supposed origin, is surprising.

It was the purpose of this study to determine the chromosome number of many of the species and some of the representative types of the commercial varieties, and, if possible, to establish the relation between the two groups. Subsequent studies will deal with chromosome behavior in interspecific and intervarietal hybrids.

PREVIOUS REPORTS

The literature on chromosome number in *Gladiolus* is not abundant. Most of the reports have given the chromosome number for only a few species or varieties. These are listed in table 1, along with the list of Ernst-Schwarzenbach (*5*), who has approached this problem along lines similar to those followed in the present investigation.

TABLE 1.—*Chromosome numbers previously reported for Gladiolus*

Species or variety	*n*	2*n*	Reported by
G. primulinus Baker:			
var. *La Meurthe*	30		De Vilmorin and Simonet (14).
var. *Priority*	14		McLean (*10*).
G. quartinianus A. Rich	14		Do.
G. quartinianus var. *Halloween*	14		Do.
G. tristis L.	14		Do.
G. tristis L. var. *concolor*		30	Ernst-Schwarzenbach (*5*).
G. cardinalis Curt		30	Do.
G. Colvillei Sweet var. *roseus*	15	30	Ernst-Schwarzenbach and Britting-ham (*5*).
G. ramosus Paxt		46	Ernst-Schwarzenbach (*5*).
G. cuspidatus Jacq	15		Do.
G. byzantinus Mill	30		Do.
G. primulinus var. *Souvenir*	30		Do.
Gladiolus varieties		30	Kinoshita (Kihara et al.) (*7*).
Do		60	Wakakuwa (Kihara et al.) (*7*).
G. gandavensis Van Houtte:			
var. *Pompée*	30		Ernst-Schwarzenbach (*5*).
var. *Alexandre*		60	Do.
var. *Red Canna*		60	Do.
G. lemoinei Hort.:			
var. *Catharina*	30		Do.
var. *Don Salluste*		60	Do.
var. *Mrs. Frank Pendleton*		60	Do.
G. nanceianus Hort. var. *desdémone*	30		Do.

[1] Received for publication July 1, 1935; issued February 1936.
[2] Reference is made by number (italic) to Literature Cited, p. 950.

It appears from table 1 that the larger summer-flowering forms are tetraploid and have a chromosome number of $60=2n$, whereas the smaller winter- or spring-flowering types, both species and varieties, are diploid and have a chromosome number of $30=2n$. One exception is *Gladiolus ramosus* (considered to be a group of hybrids) which has $46=3X+1$ chromosomes and which Ernst-Schwarzenbach (*5*) considers to be a hypertriploid.

MATERIALS AND METHODS

Seeds and corms were secured from many commercial dealers in South Africa, Europe, and the United States and, whenever possible, duplicate material was used. Some critical material was available through the kindness of Dr. F. T. McLean, of the New York Botanical Garden, and B. Y. Morrison, of the Bureau of Plant Industry, United States Department of Agriculture. Identification was made by resorting to a variety of sources, particularly to the work of Beal (*2*) and Baker (*1*). However, the ease of hybridization in *Gladiolus* makes the problem of identification a difficult one, especially where any species has been crossed with a commercial variety. McLean (*9*) reports that most of the progeny look like the species parent.

The chromosome numbers were determined chiefly from root-tip material, and some were checked in flower buds. Both were fixed with Navaschin's fluid, followed by the usual xylol-paraffin method, and stained in iron-alum haematoxylin after sectioning. Other fixing agents yielded very poor results, particularly when the chromosomes were numerous. Regardless of number, the fixation of the anthers is uncertain, a confirmation of the condition reported by Ernst-Schwarzenbach (*5*).

All drawings were made with the aid of a camera lucida and the the use of a 15× compensating ocular with a 90× 1.3 apochromatic objective.

RESULTS AND DISCUSSION

The chromosome number found in species, species hybrids, and various commercial varieties of *Gladiolus* is shown in table 2, and illustrations of the chromosomes of some representative types are presented in figure 1.

It is apparent from table 2 that the genus *Gladiolus* is heteroploid and has a basic chromosome number of 15. The majority of the species are diploid, the only exceptions being the members of the subsection Dracocephali and the European-Asiatic group. The former contains the major portion of the species involved in the supposed origin of the summer-flowering commercial varieties. The latter group has always been considered a very distinct one, and this is further indicated by the chromosome number.

The size of the chromosomes, regardless of the number, is almost the same. In fact, any variation might well be covered in one root tip. It is possible at times to detect 2 or 4 large chromosomes but the results are not consistent. Brittingham (*3*) has also called attention to this fact, and it seems probable that it may account for the counts of 31 found in occasional cells by Ernst-Schwarzenbach (*5*).

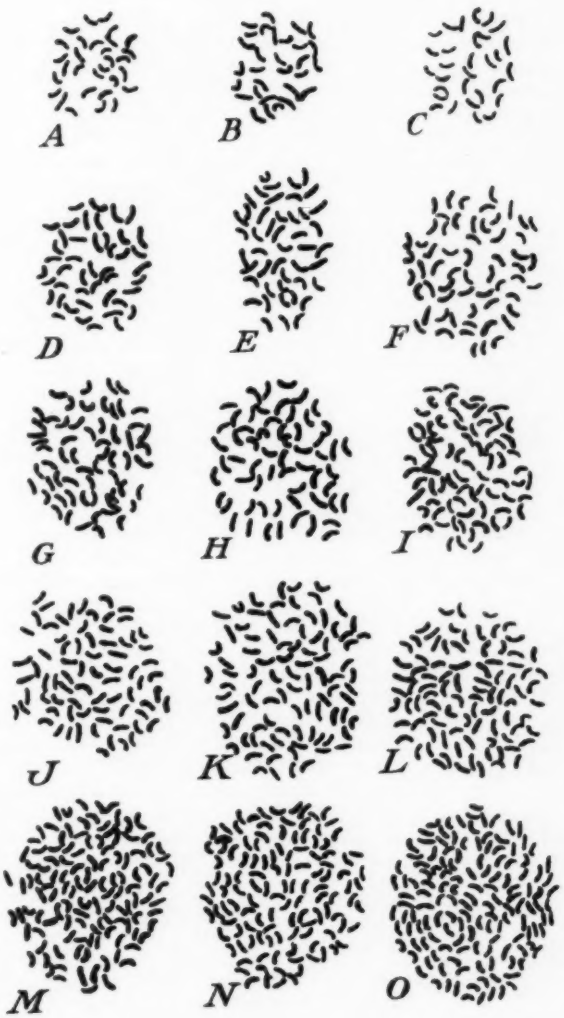

FIGURE 1.—Somatic chromosome plates from root-tip cells of *Gladiolus*: *A*, *G. cardinalis*, 2*n*=30; *B*, *G. oppositiflorus*, 2*n*=30; *C*, *G. saundersii*, 2*n*=30; *D*, *G. saundersii* hybrid, 2*n*=45; *E*, *Gladiolus* var. Nymph, 2*n*=45; *F*, *G. primulinus*, 2*n*=60; *G*, *G. platyphyllus*, 2*n*=60; *H*, *Gladiolus* var. Mr. W. H. Phipps, 2*n*=60; *I*, *G. dracocephalus* hybrid, 2*n*=75; *J*, *G. quartinianus* hybrid, 2*n*=75; *K*, *G. psittacinus*, 2*n*=90; *L*, *G. byzantinus*, 2*n*=90; *M*, *G. segetum*, 2*n*=120; *N*, *G. anatolicus*, 2*n*=120; *O*, *G. communis*, 2*n*=138±. ×2,150.

TABLE 2.—*Chromosome numbers determined for gladiolus species, species hybrids and various commercial varieties*

SPECIES AND SPECIES HYBRIDS

Name	*n*	2*n*
Eugladiolus:		
Species of Europe and western Asia:		
G. byzantinus Mill.		90
G. communis L. variety		138±
G. segetum Ker		120
G. atroviolaceous Boiss.	45	90
G. anatolicus Van Tub.		120
G. ————		60
Species of Cape and tropical Africa:		
G. tristis L.	15	30
G. tristis var. *concolor* Salis.	15	30
G. grandis Thunb.		30
G. recurvus L.		30
G. recurvus hybrid		30
G. gracilis Jacq.		30
G. angustus L.		30
G. cuspidatus Jacq.	15	30
G. trichonemifolius Ker.		30
G. brevifolius Jacq.		30
G. debilis Ker		30
G. pappei Baker		30
G. villosus Ker		30
Parviflori:		
G. crassifolius Baker		30
G. papilio Baker hybrid		75
Eugladiolus—Continued		
Blandi:		
G. blandus Aiton		30
G. hirsutus Jacq.		30
G. oppositiflorus Herb.		30
G. undulatus Jacq.	15	30
G. odoratus L. Bolus		30
G. callistus F. Bolus		30
Cardinales:		
G. cardinalis Curt.		30
G. splendens Baker		30
G. carmineus Wright		30
Dracocephali:		
G. dracocephalus Hook.		90
G. dracocephalus hybrid		75
G. psittacinus Hook.	45	90
G. psittacinus hybrid		75
G. primulinus Baker	30	60
G. platyphyllus Baker		60
G. coccineus L. Bolus		60
G. quartinianus A. Rich. hybrid		75
G. saundersii Hook.		30
G. saundersii hybrid		45
Hebea:		
G. alatus L.	15	30
G. alatus hybrid		45
G. orchidiflorus Andr. hybrid		45
G. formosus Klatt hybrid		45
G. permeabilis De la Roche		30

COMMERCIAL VARIETIES, WINTER-FLOWERING TYPES

Name	2*n*
Gladiolus colvillei Hort.:	
var. *alba*	30
var. *roseus*	30
var. *rubra*	30
G. tuberginii Hort.:	
var. Charm	45
var. Prunella	45
G. nanus Hort.:	
var. Siren	30
var. Spitfire	30
var. *cardinalis elegans*	30
var. Ackermanni	30
var. Peach Blossom	30
Gladiolus nanus Hort.—Continued.	
var. Robinhood	30
var. Blushing Bride	30
var. Nymph	45
var. Liberty	45
var. Groenendaal	60
var. Roos van Dekama	60
Herald gladiolus:	
var. Dillenberg	60
var. Joost v. d. Vondel	60
var. P. C. Hooft	60
var. Prof. Donders	60
var. Leeuwenhoek	45

COMMERCIAL VARIETIES, SUMMER-FLOWERING TYPES, (2*n*=60)

Abbé Raucourt	Commodore	King of Oranges	Pacha
Aida	Contemplation	L. von Beethoven	Pfitzer's Triumph
Albatross	Desdémone	Los Angeles	Picardy
Alice Tiplady	Dr. F. E. Bennett	Marshal Foch	Pierian
Alsace Lorraine	Encelade	Mary Jane	Porthos
Altar	Enchantress	Meadow Lark	Pride of Wanakah
Anthony Kunderd	Evelyn Kirtland	Minuet	Princeps
Ave Maria	Francis King	Mr. Mark	Prof. E. H. Wilson
Baron Joseph Hulot	Giant Nymph	Mr. W. H. Phipps	Purple Glory
Blue Isle	Gloriana	Mrs. F. C. Peters	Rob Roy
Blue Triumphator	Golden Dream	Mrs. Frank Pendleton	Rose
Break o'Day	Golden Measure	Mrs. Leon Douglas	Syncopation
Cardinal Prince	Hyperion	Mrs. P. W. Sisson	Taurus
Catharina	Impressario	Mrs. Van Konynenburg	Vermillion
Catherine Coleman	Indian Chief	October	
Cattleya Rose	Jane Addams		

While many of the records concerning the development of the commercial varieties of *Gladiolus* may be questionable, there seems to be no doubt that many of the external characters which distinguish some of the species are found in the current commercial types. McLean (*10*) and others, have shown specific cases and listed the probable species concerned. While the list in table 2 does not include all of these species, it does contain many of the key types, namely, *G. cardinalis*, *G. oppositiflorus*, *G. Saundersii*, *G. primulinus*, *G. dracocephalus*, and *G. psittacinus* and their chromosome numbers are given as 30, 60, and 90. If such species, with different chromosome numbers, are parents of the commercial varieties, it is hard to see how all of these hybrids are tetraploids. However, the occasional triploids and pentaploids might form an intermediate step in this development.

Within the past few years the attention of cytologists has been centered on the nature of the chromosomes rather than on the number, and special consideration has been given to the spindle-fiber attachment, to the satellites, and to size differences. These features have been helpful in tracing the ancestry of existing types. This method of tracing ancestry would be exceedingly difficult to apply in the case of *Gladiolus* because of the smallness of the chromosomes, so other methods must be employed. One of these seems to be a careful observation of the behavior of the triploids and pentaploids, in regard to both chromosome number and genetical characters, when they are used in crosses. That such triploids and pentaploids exist has already been pointed out. These are probably diploid-tetraploid and tetraploid-hexaploid hybrids, and recent crosses between forms with known chromosome numbers have shown that such crosses are very readily secured. If such triploids and pentaploids are again used in backcrosses, particularly as the pollen parent, selection by hybridizers from the resulting offspring might tend to be centered around those with a tetraploid number, because of certain desirable qualities which they possess. This would be especially true when a tetraploid is used as the seed parent. That the gametes of triploids and pentaploids which must effect fertilization are likely to be euploid has been shown in *Zea-Euchlaena*, *Triticum*, and *Nicotiana* hybrids and in many other crosses of this type. Longley, who has listed and discussed these hybrids (*8, p. 802*) says:

> Where the chromosome complement of a plant is made up of chromosomes in addition to the two homologous sets [triploids and pentaploids], the tendency of the functioning gametes to have the basic chromosome number or a multiple of this number must lead to the production of plants with chromosome numbers in multiples of the basic number and to the absence of plants with aneuploid chromosome numbers.

No aneuploid *Gladiolus* has yet been found except a variety of *G. communis*, and this group has apparently not entered into the formation of tetraploid summer gladiolus.

A detailed description of meiosis, particularly in the commercial varieties, is omitted from this account because of the confusing trivalents and tetravalents which need further study.

SUMMARY

The basic chromosome number of the genus *Gladiolus* is 15.

Diploid, triploid, tetraploid, pentaploid, hexaploid, octoploid, and hyperenneaploid species and hybrids have been found. The majority of species are diploid and all of the summer-flowering commercial varieties which were studied are tetraploid.

The subsection Dracocephali and the European-Asiatic group contain most of the polyploids.

A brief discussion of the possible origin of the tetraploids, in view of their parentage, is presented.

The chromosomes are small and of approximately the same size.

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